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(57) Abstract

The invention relates to ribonucleases derived from a native ribonuclease found in the oocytes of *Rana pipiens*. Various humanized and recombinant forms of these molecules are described as well as uses for them.

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RECOMBINANT RIBONUCLEASE PROTEINS

FIELD OF THE INVENTION

This invention relates to the production of ribonuclease molecules which are toxic to cells of interest.

BACKGROUND OF THE INVENTION

Ribonucleases such as ribonuclease A ("RNase A") and their cytotoxicity toward tumor cells are well documented from studies performed in the 1960s and 1970s and reviewed in Roth, J., 1963, *Cancer Res.* 23:657-666. Human serum was also discovered to contain several RNases (Reddi, E., 1975, *Biochem. Biophys. Res. Commun.* 67:110-118, Blank *et al.*, Human body fluid ribonucleases: detection, interrelationships and significance 1-203-209 (IRL Press, London, 1981)) that are expressed in a tissue specific manner. The proteins involved in the host defense activity of the eosinophil are homologous to RNases and express RNase activity (Gleich *et al.*, 1986, *Proc. Natl. Acad. Sci., USA* 83:3146-3150; Slifman *et al.*, 1986, *J. Immunol*, 137:2913-2917). Thus, human serum RNases were believed to also have host defense activities.

Further to these early studies was the discovery that an anti-tumor protein from oocytes of *Rana pipiens* has homology to RNase A (Ardelt *et al.*, 1991, *J. Biol. Chem.* 256:245-251). This protein has been termed ONCONASE®, Alfacell Corporation, N.J. See also *e.g.*, Darzynkiewicz *et al.* (1988) *Cell Tissue Kinet.* **21**, 169-182, Mikulski *et al.* (1990) *Cell Tissue Kinet.* **23**, 237-246. This protein is also described in U.S. Patent No. 4,888,172. Phase I and Phase I/II clinical trials of ONCONASE® as a single therapeutic agent in patients with a variety of solid tumors (Mikulski *et al.* (1993) *Int. J. of Oncology* **3**, 57-64) or combined with tamoxifen in patients with advanced pancreatic carcinoma have

recently been completed (Chun *et al.* (1995) *Proc Amer Soc Clin Oncol* **14 No. 157**, 210). Conjugation of ONCONASE® to cell-type-specific ligands increased its potency towards tumor cells (Rybak *et al.*(1993) *Drug Delivery* 1, 3-10). Taken together, these results indicate that ONCONASE® has properties that are advantageous for the generation of a potent selective cell killing agent.

However, since this is not a human-derived protein, it is prone to stimulating undesirable immune responses when used in humans. Thus, it would be desirable to retain the potent cytotoxic properties of this molecule while reducing its immunogenicity in humans. Further, it would be desirable to produce derivations of this molecule recombinantly so that it may be better chemically conjugated or recombinantly joined to other molecules for targeting to specific cells. Until the invention described herein, it has proven difficult to recombinantly express an active cytotoxic molecule related to ONCONASE®. Though it was thought that the methionine-glutamic acid amino terminal end of the recombinant molecule prohibited the molecule from having significant enzymatic activity, a means to solve this problem has not been forthcoming until the invention herein.

Further, although advances in protein design techniques promise to alleviate some of the immunogenicity associated with the antibody portion of immunotoxins (Bird *et al.*, 1988, *Science* 242:423; Huston *et al.*, 1988, *Proc Natl Acad Sci USA* 85:5879; Ward *et al.*, 1989, *Nature* 341:544), no solution has been forthcoming for the immunogenicity of the toxin portion other than immunosuppression of the patients (Khazaeli *et al.*, 1988, *Proceedings of AACR* 29:418). Thus, there is a continuing need for methods and compositions that would reduce the immunogenicity of the *Rana pipiens*-derived toxic moiety.

Non-cytotoxic *human* members of the RNase A superfamily linked to tumor associated antigens by chemical (Rybak *et al.*(1991) *J. Biol. Chem* **266**, 1202-21207, Newton *et al.* (1992) *J. Biol. Chem.* **267**, 19572-19578) or recombinant means (Rybak *et al. Proc. Natl. Acad. Sci. U.S.A.* **89**, 3165,

Newton et al. (1994) *J Biol Chem.* **269**, 26739-26745 have been shown to offer a strategy for selectively killing tumor cells with less immunogenicity than current strategies employing plant and bacterial toxins Rybak, S.M. & Youle, R.J. (1991) *Immunol. and Allergy Clinics of North America* **11:2**, 359-380. Human-derived ribonucleases of interest include eosinophil-derived neurotoxin (EDN) and angiogenin.

SUMMARY OF THE INVENTION

We have discovered how to construct RNases which are highly cytotoxic and which are modifications of the native ONCONASE® (nOnc). When the nOnc was expressed recombinantly it was not found to have significant cytotoxicity. Our modified versions (rOnc), however, are highly cytotoxic and otherwise retain the advantages of the native ONCONASE® molecules, while in some cases they also have increased cytotoxic properties. The rOnc molecules may be used alone or conveniently used to form chemical conjugates, as well as to form targeted recombinant immunofusions. These rOnc molecules can be used to decrease tumor cell growth. An effective recombinant form of nOnc advantageously permits the recombinant molecule to be fused to other therapeutic or targeting molecules of interest recombinantly. Further, the rOnc molecule can be modified to enhance cytotoxicity as will be seen below. Our nOnc-derived molecules are also desirable because nOnc is a unique ribonuclease in that it can be administered alone directly to patients to decrease and inhibit tumor cell growth without the use of a targeting agent.

The present invention also includes methods of selectively killing cells using a rOnc joined to a ligand to create a selective cytotoxic reagent of the present invention. The method comprises contacting the cells to be killed with a cytotoxic reagent of the present invention having a ligand binding moiety that specifically delivers the reagent to the cells to be killed. This method of the present invention may be used for cell separation *in vitro* by selectively killing unwanted types of cells, for example, in bone marrow prior to

transplantation into a patient undergoing marrow ablation by radiation, or for killing leukemia cells or T-cells that would cause graft-versus-host disease. The toxins can also be used to selectively kill unwanted cells in culture.

Humanized versions of our rOnc molecules are also described which graft portions of mammalian or human-derived RNases such as angiogenin or human eosinophil derived neurotoxin (EDN) to the rOnc-derived molecules. A preferred embodiment of the invention is a molecule where the amino terminal end of EDN is placed onto the amino terminal end of the rOnc molecules. The surprising properties of these hybrid proteins with regard to ribonuclease activity and *in vitro* anti-tumor effects are described.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE LEGENDS

Figure 1 shows the deduced amino acid sequence of the *Rana* clone 9 (SEQ ID NO:2), described below and sequence alignment with the amino acid sequence of nOnc (SEQ ID NO:1). The bold print indicates identical residues between nOnc and *Rana* clone 9. The dots indicate missing amino acids in the PCR clone.

Figures 2A and 2B show the configuration of the DNA constructs exemplified in the examples. The PCR product obtained from *Rana pipiens* DNA is identified as *Rana* 9. The N-and C-termini are synthetically filled in and identified as Onc in the constructs encoding [Met-(-1)]rOnc or EDN for the N-terminal EDN/Onc hybrid. Corresponding amino acid residues are indicated below each construct. Figure 2B shows the sequence alignment of the N-terminal sequences of nOnc (SEQ ID NO:3), rEDN (SEQ ID NO:4), [Met-(-1)]rOnc containing a Gly (G) instead of Asp in position 20 (SEQ ID NO:5), $rEDN_{(1-21)}rOnc$ with an Asp in amino acid position 26 (SEQ ID NO:6) and $rEDN_{(1-21)}rOncG26$ with a Gly in position 26 (SEQ ID NO:7). Bold letters indicate conserved residues, capital letters show the sequence deduced from *Rana* clone 9.

Figures 3A-3D show the inhibition of protein synthesis in human tumor cells by nOnc, rEDN, [Met-(-1)]rOnc or hybrid proteins. Cells (10^4) were plated in individual 96-well microtiter culture plates and treated with varying concentrations of each agent for 48 h. Cell viability was determined as described in the Example Section below. Results from more than one individual experiment were combined to yield the mean data points. Standard errors of the means, when they are greater than the symbol, are shown. Cell lines: ACHN, renal cancer (Fig.3A); MDA-MB-231 (Fig. 3B) and HS 578T (Fig.3D), breast cancer; SF-539 (Fig. 3C), CNS, cancer. EDN (open triangles); nOnc (open squares); [Met-(-1)]rOnc, (solid triangles); $rEDN_{(1-21)}$rOnc, (open circles); $rEDN_{(1-21)}$rOncG26(solid circles).

Figure 4 shows a sequence alignment of some members of the RNase A superfamily: Frog lectin is from *Rana catesbeiana*, ONCONASE®, EDN, ECP (human eosinophil cationic protein), Ang is bovine angiogenin, Seminal is bovine seminal RNase, and RNase A is bovine pancreatic RNase A (SEQ ID NOs:8, 1 and 9-13, respectively). Amino acids conserved in all members are capitalized, and active site residues H12, K41, and H119 (RNase A numbering) are marked with an asterisk.

Figure 5 shows the inhibition of protein synthesis by MetSerOnc and MetSer- or MetGlu-OncFvs. The cytotoxic effect of the single chain antibody rOnc fusion proteins; E6FB[Met-(-1)]SerrOnc (closed circles), [Met-(-1)]SerrOnc-AngFBE6 (open squares) and [Met-(-1)]GlurOncFBE6 (closed squares) were compared to the non-targeted recombinant protein, [Met-(-1)]SerrOnc (open circles), by determining inhibition of protein synthesis in SF 539 cells. Cells were plated into 96-well microtiter plates in Dulbecco's minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum. Additions were made in a total volume of 10 μl and the plates were incubated at 37° for 3 days. Phosphate buffered saline containing 0.1 mCi of [^{14}C]leucine was added for 2-4 h and the cells were harvested onto glass fiber

filters using a PHD cell harvester, washed with water, dried with ethanol and counted. The results were expressed as per cent of [^{14}C]leucine incorporation in the mock-treated wells.

Figures 6A and **6B** show inhibition of protein synthesis in an assay as described for Figures 3A-3D using cell line SF539, human glioma cells and rOnc fusion proteins designated MetLysTryrOnc (open circles, Fig. 6A); MetAlaAlaTyrOnc (closed circles, Fig. 6A); and rOnc fusion proteins with signal peptides, MetKDELSerrOnc (open circles, Fig. 6B) and MetNLSSerrOnc (closed circles Fig. 6B).

Figure 7 shows inhibition of protein synthesis in an assay as described for Figures 3A-3D using cell line SF539, human glioma cells and comparing three fusion proteins corresponding to MetSerOnc (SEQ ID NO:39 with a Met-Ser amino terminal end): MetSerOnc (closed circles), MetSerOncC4 (MetSerOnc with a Cys at amino acid position 5 of SEQ ID NO:39, closed squares) and MetSerOncC72 (MetSerOnc with a Cys at amino acid position 73 of SEQ ID NO:39, open circles).

DETAILED DESCRIPTION

This invention provides highly active and cytotoxic ribonuclease molecules which can be used to selectively kill and target cells, particularly tumor cells. In some embodiments the molecules are designed to incorporate sequences from human derived ribonucleases which are also highly active and cytotoxic, but which have the further advantage in that they are less immunogenic in humans. The rOnc molecules of the present invention are those which are recombinant nOnc-derived sequences.

The nOnc molecule has an amino acid sequence set forth in SEQ ID NO:1. Bovine pancreatic RNase A has an amino acid sequence set forth in SEQ ID NO:13. Unless otherwise indicated, the amino acid sequence positions described herein use as a frame of reference the bovine pancreatic RNase A sequence in SEQ ID NO:13 as this is the reference sequence

commonly used in the RNase field. It should be understood that such position designations do not indicate the number of amino acids in the claimed molecule *per se*, but indicate where in the claimed molecule the residue occurs when the claimed molecule sequence is aligned with bovine RNase.

The rOnc molecules described and claimed herein will preferably have cysteine residues at amino acid positions corresponding to amino acid positions 26, 40, 58, 84, 95 and 110; a lysine at position 41 and a histidine at position 119 with reference to the bovine RNase A, SEQ ID NO:13 (such positions correspond to amino acid position numbers 19, 30, 48, 68, 75 and 90 and 87 and 104 of the nOnc sequence respectively set out in SEQ ID NO:1).

The rOnc molecules of this invention are those that have measurable ribonuclease activity, as defined below. The ribonucleases will also have (a) an amino terminal end beginning with a methionine which is followed by any amino acid other than glutamic acid (Glu); (b) a cysteine at amino acid positions 26, 40, 58, 84, 95 and 110; a lysine at position 41 and a histidine at position 119, such positions being determined with reference to those in the amino acid sequence of bovine RNase A (SEQ ID NO:13), and (c) an nOnc-derived amino acid sequence.

Preferably, the rOnc molecules will have an amino terminal end selected from the group consisting of:

Met-Ala;
Met-Ala-Ala-Ser;
Met-Arg;
Met-(J);
Met-Lys-(J);
Met-Arg-(J);
Met-Lys;
Met-Lys-Pro;
Met-Lys-(J)-Pro (SEQ ID NO:14);
Met-Lys-Pro-(J) (SEQ ID NO:15);
Met-Asn;
Met-Gln;
Met-Asn-(J);

Met-Gln-(J);
Met-Asn-(J)-Pro (SEQ ID NO:16);
Met-(J)-Lys;
Met-(J)-Lys-Pro (SEQ ID NO:17); and
Met-(J)-Pro-Lys (SEQ ID NO:18);

where (J) is Ser, Tyr or Thr.

Further, it is preferred that the rOnc molecules be modified so that the aspartic acid of amino acid position 2 of nOnc (position 4 with reference to the sequence of bovine RNase A) is deleted or replaced by Ala or Asn.

In alternative forms of the rOnc molecules, the molecules will employ an amino terminal end encoded by a sequence derived from the amino terminal end of EDN followed by a sequence from rOnc. In such forms, it is preferred that the amino acid sequence is one selected from the group consisting of those sequences substantially identical to those of a formula:

$$\text{Met}(-1)\text{EDN}_{(1-m)}\text{Onc}_{(n-104)}$$

wherein Met(-1) refers to an amino terminal residue of Met; wherein $\text{EDN}_{(1-m)}$ refers to a contiguous sequence of amino acids of a length beginning at amino acid position 1 of EDN (SEQ ID NO:9) and continuing to and including amino acid position "m" of EDN; wherein $\text{Onc}_{(n-104)}$ refers to a sequence of contiguous amino acids beginning at amino acid position "n" and continuing to and including amino acid position 104 as set out in SEQ ID NO:1; such that:

when m is 21, n is 16 or 17;
when m is 22, n is 17;
when m is 20, n is 16;
when m is 19, n is 15;
when m is 18, n is 14;
when m is 17, n is 12 or 13;
when m is 16, n is 11, 12, 13 or 14;
when m is 15, n is 10;
when m is 14, n is 9;
when m is 13, n is 8; and
when m is 5, n is 1.

In other alternative embodiments, the rOnc molecule will be fused at the carboxyl end to a sequence from angiogenin, such as the sequence exemplified in SEQ ID NO:11 or that at amino acid positions 101 to 107 of SEQ ID NO:20. The nucleic acid sequence for human angiogenin is known and is set out in U.S. Patent Application No. 08/125,462.

Preferred rOnc nucleic acid sequences are those that encode preferred rOnc amino acid sequences which are substantially identical to those in SEQ ID NOs:20, 22, 24, 26, 28 and 30 (corresponding nucleic acid sequences are set out in SEQ ID NOs:19, 21, 23, 25, 27 and 29, respectively). Most preferred rOnc amino acid sequences are those that are substantially identical to those set forth in SEQ ID NOs:20, 22, 24 and 26. Their corresponding nucleic acid sequences are also preferred and are set out in SEQ ID NOs:19, 21, 23 and 25, including conservatively modified variants thereof. The most preferred sequence includes SEQ ID NO:22, one which employs an amino terminal end comprising 1 to 21 (typically 21) amino acids of the amino terminal end of EDN grafted on to 16 to 104 amino acids of the nOnc sequence, with amino acid residue 20 in nOnc (Asp) being replaced with Gly. Preferred rOnc sequences further will contain optionally a Cys at a position corresponding to amino acid position 5, or 73 or Ala at amino acid position 88 in place of Cys with reference to SEQ ID NO:39.

Comparisons of the rOnc sequences provided here can be made to described sequences in the pancreatic RNase A superfamily. Many of such members are known and include, but are not limited to, frog lectin from Rana *catesbeiana* (Titani *et al.*, *Biochemistry* 26:2189 (1987)); ONCONASE® (Ardelt, W. *et al.*, *J. Biol. Chem.* 266:245 (1991)); eosinophil derived neurotoxin (EDN) (Rosenberg *et al.*, *supra*); human eosinophil cationic protein (ECP) (Rosenberg *et al.*, *J. Exp. Med.* 170:163 (1989)); angiogenin (Ang) (Fett, J.W. *et al.*, *Biochemistry* 24:5480 (1985)); bovine seminal RNase (Preuss *et al.*, *Nuc. Acids. Res.* 18:1057 (1990)); and bovine pancreatic RNase (Beintama *et al.*, *Prog. Biophys. Mol. Biol.* 51:165 (1988)), references for all such proteins are incorporated by reference

herein. Amino acid sequence alignment for such RNases are also set out in Fig. 4 and in Youle *et al.*, *Crit. Rev. Ther. Drug. Carrier Systems* 10:1-28 (1993) and in U.S. Patent Application Serial No. 08/125,462, which is incorporated by reference herein.

Definitions.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.* (1994) *Dictionary of Microbiology and Molecular Biology*, second edition, John Wiley and Sons (New York), and Hale and Marham (1991) *The Harper Collins Dictionary of Biology,* Harper Perennial, NY provide one of skill with a general dictionary of many of the terms used in this invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined below.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

The terms "measurable ribonuclease activity" or "significant ribonuclease activity" refer to a molecule which has an IC_{50} (ng/ml) of less than 40 when added to a rabbit reticulocyte lysate assay wherein protein synthesis is inhibited as measured by the incorporation of $[^{35}S]$methionine into acid precipitable protein. IC_{50} is the concentration of protein necessary to inhibit protein synthesis by 50% in the assay. The lysate assay may be done as described in the Promega lysate assay kit which is commercially available from Promega Corporation, Madison, WI. Ribonuclease activity using high molecular weight RNA and tRNA is determined at 37°C through the formation of perchloric acid soluble nucleotides following published protocols (Newton, D.L., *et al.* (1996)

Biochemistry 35:545-553). With poly(A,C) UpG and poly U, ribonuclease activity is assayed according to DePrisco *et al.*, and Libonati and Floridi (DePrisco, R., *et al.* (1984) *Biochimica et Biophysica Acta* 788:356-363; Libonati, M. *et al.* (1969) *European J. Biochem.* 8:81-87). Activity is assayed by measuring the increase with time in absorbance at 260 nm. Incubation mixtures (1 ml of 10 mM imidazole, 0.1 M NaCl, pH 6.5 or pH 7) contain substrate and appropriate amounts of enzyme solution at 25°C. The *in vitro* translation assay (St. Clair, D.K., *et al.* (1987) *Proc. Natl. Acad. Sci.* 84:8330-8334) and the cell viability assays using the (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue) (MMT) (Mossman, T. (1983) *J. Immunol. Methods* 65:55-63) are performed as previously described (Pearson, J.W., *et al.* (1991) *J. Natl. Cancer Inst.* 83:1386-1391).

An "nOnc-derived" amino acid sequence is one that contains at least one string of six contiguous amino acids which is identical to a contiguous sequence of six amino acids selected from the group of sequences beginning at amino acid positions 1 (with Glu replacing pyroGlu), 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 41, 42, 43, 44, 45, 46, 47, 50, 52, 54, 56, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 76, 80, 81, 82, 84, 85, 86, 87, 91, 92, 93, 95, or 96 of the nOnc amino acid sequence (SEQ ID NO:1).

"Conservatively modified variations" of a particular nucleic acid sequence refer to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid

variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence. Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton (1984) Proteins W.H. Freeman and Company.

The terms "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment. The rOncs described herein are isolated and biologically pure since they are recombinantly produced in the absence of unrelated *Rana pipiens* proteins. They may, however, include heterologous cell components, a ligand binding moiety, a label and the like.

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence includes the complementary sequence thereof. A nucleic acid encodes another nucleic acid where it is the same as the specified nucleic acid, or complementary to the specified nucleic acid.

An "expression vector" includes a recombinant expression cassette which includes a nucleic acid which encodes a rOnc polypeptide which can be transcribed and translated by a cell. A recombinant expression cassette is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements which permit transcription of a particular nucleic acid in a target cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of the expression vector includes a nucleic acid to be transcribed, and a promoter.

The term "recombinant" when used with reference to a protein indicates that a cell expresses a peptide or protein encoded by a nucleic acid whose origin is exogenous to the cell. Recombinant cells can express genes that are not found within the native (non-recombinant) form of the cell. Recombinant cells can also express genes found in the native form of the cell wherein the genes are re-introduced into the cell by artificial means, for example under the control of a heterologous promoter.

The term "subsequence" in the context of a particular nucleic acid or polypeptide sequence refers to a region of the nucleic acid or polypeptide equal to or smaller than the particular nucleic acid or polypeptide.

"Stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence dependent,

and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York. Generally, highly stringent wash conditions are selected to be about 5° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and ph. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m point for a particular probe. Nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

The term "identical" in the context of two nucleic acid or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins or peptides it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a

non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, *e.g.*, according to the algorithm of Meyers and Miller, Computer Applic. Biol. Sci., 4: 11-17 (1988) *e.g.*, as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482; by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443; by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444; by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA); the CLUSTAL program is well described by Higgins and Sharp (1988) *Gene*, 73: 237-244 and Higgins and Sharp (1989) *Computer Applications in the Biosciences* 5: 151-153; Corpet, *et al.* (1988) *Nucleic Acids Research* 16, 10881-90; Huang, *et al.* (1992) *Computer Applications in the Biosciences* 8, 155-65, and Pearson, *et al.* (1994) *Methods in Molecular Biology* 24, 307-31. Alignment is also often performed by inspection and manual alignment.

The term "substantial identity" or "substantial similarity" in the context of a polypeptide indicates that a polypeptide comprises a sequence with at least 70% sequence identity to a reference sequence, or preferably 80%, or more preferably 85% sequence identity to the reference sequence, or most preferably 90% identity over a comparison window of about 10-20 amino acid residues. An indication that two polypeptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a polypeptide is substantially

identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

One indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. Stringent conditions are sequence dependent and are different under different environmental parameters. Generally, stringent conditions are selected to be about 5° C to 20°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

The term "specifically deliver" as used herein refers to the preferential association of a molecule with a cell or tissue bearing a particular target molecule or marker and not to cells or tissues lacking that target molecule. It is, of course, recognized that a certain degree of non-specific interaction may occur between a molecule and a non-target cell or tissue. Nevertheless, specific delivery, may be distinguished as mediated through specific recognition of the target molecule. Typically specific delivery results in a much stronger association between the delivered molecule and cells bearing the target molecule than between the delivered molecule and cells lacking the target molecule. Specific delivery typically results in greater than 2 fold, preferably greater than 5 fold, more preferably greater than 10 fold and most preferably greater than 100 fold increase in amount of delivered molecule (per unit time) to a cell or tissue bearing

the target molecule as compared to a cell or tissue lacking the target molecule or marker.

The term "residue" as used herein refers to an amino acid that is incorporated into a polypeptide. The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

A "fusion protein" or when a molecule is "joined" to another refers to a chimeric molecule formed by the joining of two or more polypeptides through a peptide bond formed between the amino terminus of one polypeptide and the carboxyl terminus of another polypeptide. The fusion protein or the joined molecules may be formed by the chemical coupling of the constituent molecules or it may be expressed as a single polypeptide from a nucleic acid sequence encoding a single contiguous fusion protein. A single chain fusion protein is a fusion protein having a single contiguous polypeptide backbone.

A "ligand" or a "ligand binding moiety", as used herein, refers generally to all molecules capable of specifically delivering a molecule, reacting with or otherwise recognizing or binding to a receptor on a target cell. Specifically, examples of ligands include, but are not limited to, antibodies, lymphokines, cytokines, receptor proteins such as CD4 and CD8, solubilized receptor proteins such as soluble CD4, hormones, growth factors, and the like which specifically bind desired target cells.

Making rOnc-derived Nucleic Acids and Polypeptides.

Several specific nucleic acids encoding rOnc-derived polypeptides are described herein. These nucleic acids can be made using standard recombinant or synthetic techniques. Given the nucleic acids of the present invention, one of skill can construct a variety of clones containing functionally equivalent nucleic acids, such as nucleic acids which encode the same polypeptide. Cloning methodologies to accomplish these ends, and sequencing methods to verify the sequence of

nucleic acids are well known in the art. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology* volume 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3; and *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel). Product information from manufacturers of biological reagents and experimental equipment also provide information useful in known biological methods. Such manufacturers include the SIGMA chemical company (Saint Louis, MO), R&D systems (Minneapolis, MN), Pharmacia LKB Biotechnology (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersberg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen, San Diego, CA, and Applied Biosystems (Foster City, CA), as well as many other commercial sources known to one of skill.

The nucleic acid compositions of this invention, whether RNA, cDNA, genomic DNA, or a hybrid of the various combinations, are isolated from biological sources or synthesized *in vitro*. The nucleic acids of the invention are present in transformed or transfected cells, in transformed or transfected cell lysates, or in a partially purified or substantially pure form.

In vitro amplification techniques suitable for amplifying sequences for use as molecular probes or generating nucleic acid fragments for subsequent subcloning are known. Examples of techniques sufficient to direct persons of skill through such *in vitro* amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques (*e.g.*, NASBA) are found in Berger,

Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd Ed) Vol. 1-3; and Ausubel, as well as Mullis *et al.*, (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN* 36-47; *The Journal Of NIH Research* (1991) 3, 81-94; (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86, 1173; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87, 1874; Lomell *et al.* (1989) *J. Clin. Chem* 35, 1826; Landegren *et al.*, (1988) *Science* 241, 1077-1080; Van Brunt (1990) *Biotechnology* 8, 291-294; Wu and Wallace, (1989) *Gene* 4, 560; Barringer *et al.* (1990) *Gene* 89, 117, and Sooknanan and Malek (1995) *Biotechnology* 13: 563-564. Improved methods of cloning *in vitro* amplified nucleic acids are described in Wallace *et al.*, U.S. Pat. No. 5,426,039.

Oligonucleotides for use as probes, *e.g.*, in *in vitro* rOnc nucleic acid amplification methods, or for use as nucleic acid probes to detect rOnc nucleic acids are typically synthesized chemically according to the solid phase phosphoramidite triester method described by Beaucage and Caruthers (1981), *Tetrahedron Letts.*, 22(20):1859-1862, *e.g.*, using an automated synthesizer, *e.g.*, as described in Needham-VanDevanter *et al.* (1984) *Nucleic Acids Res.*, 12:6159-6168. Oligonucleotides can also be custom made and ordered from a variety of commercial sources known to persons of skill. Purification of oligonucleotides, where necessary, is typically performed by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson and Regnier (1983) *J. Chrom.* 255:137-149. The sequence of the synthetic oligonucleotides can be verified using the chemical degradation method of Maxam and Gilbert (1980) in Grossman and Moldave (eds.) Academic Press, New York, *Methods in Enzymology* 65:499-560.

One of skill will recognize many ways of generating desired alterations in a given nucleic acid sequence. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or

radiation, chemical synthesis of a desired oligonucleotide (*e.g.*, in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. *See*, Giliman and Smith (1979) *Gene* 8:81-97; Roberts *et al.* (1987) *Nature* 328:731-734 and Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd Ed) Vol. 1-3; Innis, Ausbel, Berger, Needham VanDevanter and Mullis (*all supra*).

Polypeptides of the invention can be synthetically prepared in a wide variety of well-known ways. Polypeptides of relatively short size are typically synthesized in solution or on a solid support in accordance with conventional techniques. *See, e.g.*, Merrifield (1963) *J. Am. Chem. Soc.* 85:2149-2154. Various automatic synthesizers and sequencers are commercially available and can be used in accordance with known protocols. *See, e.g.*, Stewart and Young (1984) *Solid Phase Peptide Synthesis*, 2d. ed., Pierce Chemical Co. Polypeptides are also produced by recombinant expression of a nucleic acid encoding the polypeptide followed by purification using standard techniques.

Making Conservative Modifications of the Nucleic Acids and Polypeptides of the Invention.

One of skill will appreciate that many conservative variations of the sequences disclosed yield a substantially identical rOnc. For example, due to the degeneracy of the genetic code, "silent substitutions" (*i.e.*, substitutions of a nucleic acid sequence which do not result in an alteration in an encoded polypeptide) are an implied feature of *every* nucleic acid sequence which encodes an amino acid. Similarly, conservative amino acid substitutions, in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties (*see*, the definitions section, *supra*), are also readily identified as being highly similar to a disclosed amino acid sequence, or to a disclosed nucleic acid sequence which encodes an amino acid. Such conservatively substituted (or modified) variations of each explicitly disclosed sequence are a feature of the present invention.

One of skill will recognize many ways of generating alterations in a given nucleic acid sequence. Such well-known methods include site-directed mutagenesis, PCR amplification using degenerate oligonucleotides, exposure of cells containing the nucleic acid to mutagenic agents or radiation, chemical synthesis of a desired oligonucleotide (*e.g.*, in conjunction with ligation and/or cloning to generate large nucleic acids) and other well-known techniques. *See*, Giliman and Smith (1979) *Gene* 8:81-97, Roberts *et al.* (1987) *Nature* 328:731-734 and Sambrook, Innis, Ausbel, Berger, Needham VanDevanter and Mullis (*all supra*).

Most commonly, polypeptide sequences are altered by changing the corresponding nucleic acid sequence and expressing the polypeptide. However, polypeptide sequences are also optionally generated synthetically using commercially available peptide synthesizers to produce any desired polypeptide (*see*, Merrifield, and Stewart and Young, *supra*).

One of skill can select a desired nucleic acid or polypeptide of the invention based upon the sequences provided and upon knowledge in the art regarding ribonucleases generally. The physical characteristics and general properties of RNases are known to skilled practitioners. The specific effects of some mutations in RNases are known. Moreover, general knowledge regarding the nature of proteins and nucleic acids allows one of skill to select appropriate sequences with activity similar or equivalent to the nucleic acids and polypeptides disclosed in the sequence listings herein. The definitions section herein describes exemplary conservative amino acid substitutions.

Finally, most modifications to nucleic acids and polypeptides are evaluated by routine screening techniques in suitable assays for the desired characteristic. For instance, changes in the immunological character of a polypeptide can be detected by an appropriate immunological assay. Modifications of other properties such as nucleic acid hybridization to a target nucleic acid, redox or thermal stability of a protein, thermal histeresis, hydrophobicity, susceptibility to

proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

rOnc Fusion Proteins and Other Therapeutic Moieties.

The rOnc molecules may also include pharmacological agents or encapsulation systems containing various pharmacological agents. They typically will include a ligand to act as a targeting molecule to direct the rOnc to desired cells. The rOnc may be attached directly to a ligand or an antisense molecule which will assist in delivering the rOnc. See, for example, SEQ ID NOS:40-61. The rOnc can also be engineered to contain a nuclear localization signal ("NLS") such as that described in amino acid positions 1 to 7 in SEQ ID NO:32 (and SEQ ID NO:31) to direct the rOnc within the cell. Alternatively, the Met at position 8 and the corresponding nucleic acids at positions 22-24 of SEQ ID NO:31 has been and can be omitted. The nucleic acid sequence for the NLS is nucleic acids 1-21 of SEQ ID NO:31. A signal peptide is also exemplified at amino acid positions 1-25 of SEQ ID NO:63.

The rOnc molecules are uniquely adapted for gene therapy applications. They can be fused to other therapeutic agents, for example, they could be fused to an anti-B cell lymphoma antibody. For example, as will be explained in more detail below, rOnc molecules recombinantly fused to an anti-transferrin receptor antibody or an anti-colon cancer antibody were active. As mentioned above, nOnc has anti-tumor effects *in vivo* and preferentially kills rapidly dividing cells stimulated by serum or growth promoting agents such as ras. The molecules are readily internalized in the cell. Their activity can be further facilitated by joining them to a nuclear localization signal and the like to redirect the molecules within the cell. Of particular use in tumor cells would be to target telomerase, an enzyme subject to degradation by RNase.

We have found that Onc synergizes with ras in microinjection studies. This means that Onc and ras have to be together in the cell. Onc does not synergize with ras when it enters the cell via its own routing. A CAAX (SEQ ID NO:33)

motif is required to localize ras at the plasma membrane (C=Cys, A = an aliphatic amino acid, X = S,M,C,A, or Q, an example is Cys-Val-Ile-Met (SEQ ID NO:34)). Importantly this type of sequence has been shown to target heterologous proteins to the plasma membrane (Hancock, J., Cadwallader, K., Paterson, H. and C. Marshall (1991) EMBO J. 10:4033). It would be desirable to join the rOnc gene with DNA encoding a CAAX (SEQ ID NO:33) signal as given in the example, or KDEL as described below.

Telomerase is being investigated as a "universal cancer target" (G.B. Morin, JNCI. (1995) 87:859). It is an RNA protein that is located in the nucleus. It has been shown that antisense to telomerase RNA can inhibit the function of the enzyme and block the growth of cancer cells (J. Feng *et al.*, *Science* (1995) 269:1236). RNase can also destroy the activity of the enzyme. Onc can also destroy the activity of the enzyme when incubated with a cell extract containing telomerase. An NLS/Onc molecule (such as that set out in SEQ ID NO: 32) can be made to route Onc to the nucleus so that it can degrade telomerase. The NLS we used has been shown to redirect proteins to the nucleus for the aim of interfering with the function of a nuclear antigen (S. Biocca, M. S. Neuberger and A. Cattaneo, (1990) 9:101). Our NLS/Onc molecule is effective in killing cells.

An amino terminal sequence to the recombinant molecule may be preferred where it is desirable to translocate the molecule into the cytosol of target cells. Such signal peptide is typically inserted at the amino end of the protein. For example, the first amino acids of the recombinant molecules described herein (after Met) could be KDEL (SEQ ID NO:64) and would accomplish signalling the molecule to the endoplasmic reticulum. Amino acid sequences which include KDEL, repeats of KDEL, or other sequences that function to maintain or recycle proteins into the endoplasmic reticulum, referred to here as "endoplasmic retention sequences" may be employed.

Optionally, the rOnc molecule attached to a ligand may include an encapsulation system, such as a liposome or

micelle that contains an additional therapeutic composition such as a drug, a nucleic acid (*e.g.* an antisense nucleic acid), or another therapeutic moiety that is preferably shielded from direct exposure to the circulatory system. Means of preparing liposomes attached to antibodies are well known to those of skill in the art. See, for example, U.S. Patent No. 4,957,735, Connor *et al.*, *Pharm. Ther.*, 28: 341-365 (1985).

One of skill will appreciate that the ligand molecule or other therapeutic component and the rOnc molecule may be joined together in any order. Thus, where the ligand is a polypeptide, the rOnc molecule may be joined to either the amino or carboxy termini of the ligand or may also be joined to an internal region of either molecule as long as the attachment does not interfere with the respective activities of the molecules.

The molecules may be attached by any of a number of means well-known to those of skill in the art. Typically the rOnc will be conjugated, either directly or through a linker (spacer), to the ligand. However, where both the rOnc and the ligand or other therapeutic are polypeptides it is preferable to recombinantly express the chimeric molecule as a single-chain fusion protein.

In one embodiment, the rOnc molecule is chemically conjugated to another molecule (*e.g.* a cytotoxin, a label, a ligand, or a drug or liposome). Means of chemically conjugating molecules are well-known to those of skill.

The procedure for attaching an agent to an antibody or other polypeptide targeting molecule will vary according to the chemical structure of the agent. Polypeptides typically contain a variety of functional groups; *e.g.*, carboxylic acid (COOH) or free amine ($-NH_2$) groups, which are available for reaction with a suitable functional group on an rOnc molecule to bind the other molecule thereto.

Alternatively, the ligand and/or rOnc molecule may be derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment

of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford Illinois.

A "linker", as used herein, is a molecule that is used to join two molecules. The linker is capable of forming covalent bonds to both molecules. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where both molecules are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (*e.g.*, through a disulfide linkage to cysteine). However, in a preferred embodiment, the linkers will be joined to the alpha carbon amino and carboxyl groups of the terminal amino acids.

A bifunctional linker having one functional group reactive with a group on a particular agent, and another group reactive with an antibody, may be used to form a desired immunoconjugate. Alternatively, derivatization may involve chemical treatment of the ligand, *e.g.*, glycol cleavage of the sugar moiety of a glycoprotein antibody with periodate to generate free aldehyde groups. The free aldehyde groups on the antibody may be reacted with free amine or hydrazine groups on an agent to bind the agent thereto. (See U.S. Patent No. 4,671,958). Procedures for generation of free sulfhydryl groups on polypeptides, such as antibodies or antibody fragments, are also known (See U.S. Pat. No. 4,659,839).

Many procedure and linker molecules for attachment of various compounds including radionuclide metal chelates, toxins and drugs to proteins such as antibodies are known. See, for example, European Patent Application No. 188,256; U.S. Patent Nos. 4,671,958, 4,659,839, 4,414,148, 4,699,784; 4,680,338; 4,569,789; and 4,589,071; and Borlinghaus *et al.* *Cancer Res.* 47: 4071-4075 (1987) which are incorporated herein by reference. In particular, production of various immunotoxins is well-known within the art and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe *et al.*, *Monoclonal Antibodies in Clinical Medicine*, Academic Press, pp. 168-190 (1982),

Waldmann, *Science*, 252: 1657 (1991), U.S. Patent Nos. 4,545,985 and 4,894,443 which are incorporated herein by reference.

In some circumstances, it is desirable to free the rOnc from the ligand when the chimeric molecule has reached its target site. Therefore, chimeric conjugates comprising linkages which are cleavable in the vicinity of the target site may be used when the effector is to be released at the target site. Cleaving of the linkage to release the agent from the ligand may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site. When the target site is a tumor, a linker which is cleavable under conditions present at the tumor site (*e.g.* when exposed to tumor-associated enzymes or acidic pH) may be used.

A number of different cleavable linkers are known to those of skill in the art. See U.S. Pat. Nos. 4,618,492; 4,542,225, and 4,625,014. The mechanisms for release of an agent from these linker groups include, for example, irradiation of a photolabile bond and acid-catalyzed hydrolysis. U.S. Pat. No. 4,671,958, for example, includes a description of immunoconjugates comprising linkers which are cleaved at the target site *in vivo* by the proteolytic enzymes of the patient's complement system. In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

Production of rOnc Molecules or Fusion Proteins

Where the molecules of interest are relatively short (*i.e.*, less than about 50 amino acids) they may be synthesized using standard chemical peptide synthesis techniques. Where two molecules of interest are relatively short the chimeric molecule may be synthesized as a single contiguous polypeptide. Alternatively the molecules may be synthesized

separately and then fused by condensation of the amino terminus of one molecule with the carboxyl terminus of the other molecule thereby forming a peptide bond. Alternatively, the molecules may each be condensed with one end of a peptide spacer molecule thereby forming a contiguous fusion protein.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is the preferred method for the chemical synthesis of the polypeptides of this invention. Techniques for solid phase synthesis are described by Barany and Merrifield, *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.*, Merrifield, *et al. J. Am. Chem. Soc.*, 85: 2149-2156 (1963), and Stewart *et al.*, *Solid Phase Peptide Synthesis, 2nd ed.* Pierce Chem. Co., Rockford, Ill. (1984) which are incorporated herein by reference.

In a preferred embodiment, the chimeric fusion proteins of the present invention are synthesized using recombinant DNA methodology. Generally this involves creating a DNA sequence that encodes the fusion protein, placing the DNA in an expression cassette under the control of a particular promoter, expressing the protein in a host, isolating the expressed protein and, if required, renaturing the protein.

DNA encoding the fusion proteins of this invention, as well as the rOnc molecules themselves, may be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences or direct chemical synthesis by methods such as the phosphotriester method of Narang *et al. Meth. Enzymol.* 68: 90-99 (1979); the phosphodiester method of Brown *et al.*, *Meth. Enzymol.* 68: 109-151 (1979); the diethylphosphoramidite method of Beaucage *et al.*, *Tetra. Lett.*, 22: 1859-1862 (1981); and the solid support method of U.S. Patent No. 4,458,066, all incorporated by reference herein.

Chemical synthesis produces a single-stranded oligonucleotide. This may be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill would recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Alternatively, subsequences may be cloned and the appropriate subsequences cleaved using appropriate restriction enzymes. The fragments may then be ligated to produce the desired DNA sequence.

In a preferred embodiment, DNA encoding fusion proteins or rOnc molecules of the present invention may be cloned using DNA amplification methods such as polymerase chain reaction (PCR). If two molecules are joined together, one of skill will appreciate that the molecules may be separated by a peptide spacer consisting of one or more amino acids. Generally the spacer will have no specific biological activity other than to join the proteins or to preserve some minimum distance or other spatial relationship between them. However, the constituent amino acids of the spacer may be selected to influence some property of the molecule such as the folding, net charge, or hydrophobicity.

The nucleic acid sequences encoding the rOnc molecules or the fusion proteins may be expressed in a variety of host cells, including *E. coli,* other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO and HeLa cells lines and myeloma cell lines. The recombinant protein gene will be operably linked to appropriate expression control sequences for each host. For *E. coli* this includes a promoter such as the T7, trp, or lambda promoters, a ribosome binding site and preferably a transcription termination signal. For eukaryotic cells, the control sequences will include a promoter and preferably an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, etc., and a polyadenylation sequence, and may include splice donor and acceptor sequences.

The expression vectors or plasmids of the invention can be transferred into the chosen host cell by well-known methods such as calcium chloride transformation for *E. coli* and calcium phosphate treatment or electroporation for mammalian cells. Cells transformed by the plasmids can be selected by resistance to antibiotics conferred by genes contained on the plasmids, such as the amp, gpt, neo and hyg genes.

Once expressed, the recombinant rOnc or fusion proteins can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (*see*, generally, R. Scopes, *Protein Purification*, Springer-Verlag, N.Y. (1982), Deutscher, *Methods in Enzymology Vol. 182: Guide to Protein Purification.*, Academic Press, Inc. N.Y. (1990)). Substantially pure compositions of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically.

Accordingly, this invention also provides for host cells and expression vectors comprising the nucleic acid sequences described above.

Further, the present invention includes a method of selectively killing cells using a rOnc joined to a ligand to create a selective cytotoxic reagent of the present invention. The method comprises contacting the cells to be killed with a cytotoxic reagent of the present invention having a ligand binding moiety that specifically delivers the reagent to the cells to be killed. This method of the present invention may be used for cell separation *in vitro* by selectively killing unwanted types of cells, for example, in bone marrow prior to transplantation into a patient undergoing marrow ablation by radiation, for killing leukemia cells or T-cells that would cause graft-versus-host disease.

For methods of use *in vivo*, preferably the mammalian protein of the reagent used in this method is endogenous to the species in which the reagent is intended for use.

Preferably, for use in humans, the cytotoxic reagent is a fusion protein comprising a humanized chimeric antibody and a humanized rOnc. Specific *in vivo* methods of this invention include a method for the chemotherapeutic alleviation of cancer in mammals comprising administering a cytotoxic amount of a selective cytotoxic reagent according to the present invention. The methods are particularly useful for treating tumors sensitive to the cytotoxic reagents. Tumors of particular interest include pancreatic, colon, breast and kidney tumors.

Pharmaceutical Compositions

The rOnc molecules and fusion proteins employing them of this invention are useful for parenteral, topical, oral, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, capsules and lozenges. It is recognized that the subject molecules and fusion proteins and pharmaceutical compositions of this invention, when administered orally, must be protected from digestion. This is typically accomplished either by complexing the protein with a composition to render it resistant to acidic and enzymatic hydrolysis or by packaging the protein in an appropriately resistant carrier such as a liposome. Means of protecting proteins from digestion are well known in the art.

The pharmaceutical compositions of this invention are particularly useful for parenteral administration, such as intravenous administration or administration into a body cavity or lumen of an organ. The compositions for administration will commonly comprise a solution of the chimeric molecule dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, *e.g.*, buffered saline and the like. These solutions are sterile and generally free of undesirable

matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of therapeutic molecule in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

Thus, a typical pharmaceutical composition for intravenous administration would be about 0.1 to 10 mg per patient per day. Dosages from 0.1 up to about 100 mg per patient per day may be used, particularly when the drug is administered to a secluded site and not into the blood stream, such as into a body cavity or into a lumen of an organ. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science*, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The compositions containing the present rOnc molecules or the fusion proteins or a cocktail thereof (*i.e.*, with other proteins) can be administered for therapeutic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease, in a cytotoxic amount, an amount sufficient to kill cells of interest. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health.

Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of

the proteins of this invention to effectively treat the patient.

All patents, applications and publications cited herein are incorporated by reference herein. The following examples are provided for illustrative purposes only and are not to be construed as limiting the invention in any way.

EXAMPLES

Example I. Cloning and Expression of rOnc and Onc conjugates with EDN.

A. Materials. Native ONCONASE® ("nOnc") (SEQ ID NO:1) Ardelt *et al.* (1991) *J. Biol. Chem.* **256**, 245-251 and recombinant human EDN ("rEDN") (SEQ ID NO:9) Newton *et al.* (1994) *J Biol Chem.* **269**, 26739-26745 were purified from *Rana pipiens* oocytes, NASCO, Fort Atkinson, WI and *Escherichia coli*, respectively, as described. Antibodies to the denatured proteins were prepared by Assay Research, Inc., College Park, MD. Reagents for performing PCR, and direct cloning of PCR products, were obtained from Perkin-Elmer Corp., Norwalk, CT and from Invitrogen, San Diego, CA respectively. Substrates for the ribonuclease assays were purchased from Sigma, St. Louis, MO and Boehringer Mannheim, Indianapolis, IN. The materials and their sources used in the construction and expression of the recombinant proteins as well as the rabbit reticulocyte lysate are described by Newton *et al.*, *Biochemistry* 35:545 (1996).

B. PCR Cloning of Onconase. *Rana pipiens* genomic DNA was isolated according to standard procedures using proteinase K Maniatis, T., Fritsch, E.F. & Sambrook, *J. Molecular Cloning, a laboratory manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982). A series of degenerate primers were designed to correspond to amino acids in various regions of the published nOnc sequence Ardelt *et al.* (1991) *J. Biol. Chem.* **256**, 245-251. The PCR reaction was performed according to the manufacturer's instructions using 15 μg of genomic DNA in 100 μl. All reagents except the DNA were combined and incubated at 95°C for 8 min to inactivate any residual proteinase K before the addition of the Taq DNA

polymerase. PCR was performed for 40 cycles of denaturation at 94°C for 1 min, annealing for 2 min at 55°C and primer extension for 2 min at 72°C. Several pairs of primers yielded products of the expected size. The largest product (252 bp) was obtained using the forward primer encoding amino acid residues 15-23 (AG(GA)GATGT(GT)GATTG(TC)GATAA(CT)ATCATG) (SEQ ID NO:35) and the reverse primer encoding amino acid residues 90-98 (TGTGA(AG)AA(CT)CAGGC(AC)CC(TA)GT(GT)CA(CT)TTT) (SEQ ID NO:36). This fragment was subcloned into pCR™II by TA cloning and a clone carrying an insert of the appropriate size was directly sequenced and found to encode amino acid residues 16-98 of nOnc ("Rana 9") (SEQ ID NO:2). The corresponding nucleic acid sequence is set out in SEQ ID NO:37.

C. **Plasmid Construction, Expression, Protein Purification and *in Vitro* Assays.** The N- and C-termini of nOnc were reconstructed using the PCR technique of splicing by overlap extension Horten *et al.* (1990) *BioTechniques* **8**, 528-532 with amino acid residues 1-15 of nOnc or amino acid residues 1-21 of EDN at the N-terminal and amino acid residues 99-104 of nOnc at the C-terminal. The assembled genes were inserted between the XbaI and BamHI sites of the bacterial expression vector, pET-11d, Novagen, Madison, WI. All procedures were accomplished essentially as described in Newton *et al.* (1994) *J Biol Chem.* **269**, 26739-26745. The plasmids were expressed in BL21(DE3) *E. coli* cells as recommended by the supplier, Novagen, Madison WI. The fusion proteins were isolated from inclusion bodies, denatured, renatured and dialyzed as described Newton *et al.* (1994) *J Biol Chem.* **269**, 26739-26745 before being applied to a CM-Sephadex C-50 column, Pharmacia Biotech Inc., Piscataway, NJ. The proteins were eluted with a NaCl gradient (0-0.5M) in 20 mM Tris-HCl, pH 7.5, containing 10% glycerol. Final purification to >95% was achieved by size exclusion chromatography on Sephadex G-100 equilibrated and eluted with 5% formic acid. The proteins were pooled, concentrated by amicon ultrafiltration using a YM3 membrane (or lyophilized), Amicon, Beverly, MA and dialyzed against 20 mM Tris-HCl, pH 7.5, containing 10% glycerol before being assayed.

Ribonuclease activity using high molecular weight RNA and tRNA was determined following published protocols, Newton *et al.* (1994) *J Neurosci* **14**, 538-544 at 37°C through the formation of perchloric acid soluble nucleotides following published protocols (Newton *et al.* (1996) *Biochem.* **35**:545-553). With poly (A,C), UpG and poly U, ribonuclease activity was assayed spectrophotometrically according to DePrisco *et al.*, and Libonati and Florida DePrisco *et al.* (1984) *Biochimica et Biophysica Acta* **788**, 356-363, Libonati, M. & Floridi, A. (1969) *European J. Biochem.* **8**, 81-87. Briefly, activity was assayed by measuring the increase in absorbance at 260 nm. Incubation mixtures (1 ml of 10 mM imidazole, 0.1 M NaCl, pH 6.5 or pH 7) contained substrate and appropriate amounts of enzyme solution at 25°C. The *in vitro* translation assay, St. Clair *et al.* (1987) *Proc Natl Acad Sci* **84**, 8330-8334, and the cell viability assays, Pearson *et al.* (1991) *J Natl Cancer Inst* **83**, 1386-1391, using the (3-[4,5-Dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide; Thiazolyl blue] (MTT) Mossman, T. (1983) *J. Immunol. Methods* **65**, 55-63 were performed as previously described.

D. Cloning and Expression of [Met-(-1)] rOnc and rOnc chimeras. Eight different oligonucleotide primers were designed to correspond to specific regions in the primary amino acid structure of nOnc, Ardelt *et al.* (1991) *J. Biol. Chem.* **256**, 245-251 and amplification of *Rana pipiens* genomic DNA was carried out in a thermal cycler, as described above. A primer pair corresponding to amino acid residues 15 to 23 and 90 to 98 of nOnc, respectively, generated a 252 bp fragment. That PCR product, here denoted *Rana* clone 9, was cloned into pCR™II and sequence analysis confirmed that the PCR product encoded Onc (104 amino acids, total) from amino acid residue 16 to 98 (Fig. 1).

The entire recombinant Onc ("rOnc") gene (SEQ ID NO:38) was constructed by PCR extension and cloned into an expression vector using methodology previously described Newton *et al.* (1994) *J Biol Chem.* **269**, 26739-26745. The amino and carboxyl termini of rOnc were completed by inserting the

first 15 and last 6 amino acid residues of nOnc, respectively. The configuration of the semi-synthetic rOnc gene is depicted at the top of Fig. 2A. The primers were designed to overlap with the DNA sequence of the *Rana* clone 9 PCR product. The plasmid was expressed in BL21(DE3) *E. coli* and the recombinant protein was isolated from inclusion bodies as described in Newton *et al.* (1994) *J Biol Chem.* **269,** 26739-26745 before being applied to a CM Sephadex C-50 column. Final purification to >95% was achieved by size exclusion chromatography. The rOnc obtained from the bacteria in this expression system contains an extra methionine at the amino terminal [Met-(-1)] (SEQ ID NO:39) in contrast to the authentic pyroglutamyl amino acid residue (<Glu-1) of the native protein (SEQ ID NO:1).

To humanize [Met-(-1)] rOnc while maintaining the alignment of the active site residues (Fig. 2B), the N-terminal of *Rana* clone 9 was also reconstituted with oligonucleotides that coded for the first 21 amino acid residues of a human eosinophil RNase, EDN (Fig. 2B, $rEDN_{(1-21)}Onc$). PCR cloning can result in sequence errors. Indeed, the DNA sequence of the gene encoding $EDN_{(1-21)}Onc$ contained an A to G substitution resulting in a change from Asp to Gly at position 26 in the chimera (residue 20 in nOnc) and is designated as $rEDN_{(1-21)}rOncG_{26}$ in Fig. 2B. Another plasmid containing encoding $rEDN_{(1-21)}rOnc$ was sequenced and found to have the correct DNA sequence. Since the mutation resulted in the substitution of a charged amino acid with a small neutral residue the mutant chimera was also expressed and characterized for activity. In addition, [Met-(-1)]rOnc was mutated at position 20 from Asp to Gly ($rOncGly_{20}$, Figs. 2A and B).

E. Ribonuclease activity of Onc, EDN, [Met-(-1)]rOnc and hybrid rOnc proteins. Both nOnc (Lin, J.J., *et al.* (1994) *Biochem Biophys Res Commun* **204**, 156-162) and EDN (Saxena *et al.* (1992) *J. Biol. Chem.* **267**, 21982-21986) are potent inhibitors of *in vitro* translation in the rabbit reticulocyte lysate by mechanisms that depend upon their respective

nucleolytic activities. As depicted in Table 1, the addition of nOnc or EDN to a rabbit reticulocyte lysate caused the inhibition of protein synthesis as measured by the incorporation of [^{35}S]methionine into acid precipitable protein. Whereas both nOnc and EDN inhibited protein synthesis with IC_{50s} of 0.2 and 1.3 ng/ml, respectively, [Met-(-1)]rOnc, [Met-(-1)]rOncG20, and $rEDN_{(1-21)}$rOncG26 were considerably less potent (IC_{50s} 98, 28 and 28 ng/ml, respectively). The least active RNase in this assay was $rEDN_{(1-21)}$rOnc with an IC_{50} of 1600 ng/ml. Placental ribonuclease inhibitor (PRI) binds tightly to EDN and inhibits its enzymatic activity, Sorrentino *et al.* (1992) *J. Biol. Chem* **267,** 14859-14865, yet nOnc activity is very little affected by PRI, Wu, Y.N., *et al.* (1993) *Journal of Biological Chemistry* **268,** 10686-10693 and Table 1, despite its homology to EDN and other members of the pancreatic RNase superfamily. In this regard, it is interesting that the activity of $rEDN_{(1-21)}$rOnc is, like nOnc, barely affected by PRI while the hybrid RNase with the Gly mutation now behaves more like EDN in that its activity is significantly inhibited (21 fold) by PRI.

The ribonuclease activity of these proteins was also assessed in assays using high and low molecular weight substrates. As shown in Table 2, EDN and nOnc have different substrate specificities consistent with previously published results (Ardelt *et al.* (1991) *J. Biol. Chem.* **256**, 245-251, Sorrentino *et al.* (1992) *J. Biol. Chem* **267,** 14859-14865, Ardelt *et al.* (1994) *Protein Sci* **3**, **Suppl. 1**, 137). Consistent with the results presented in Table 1, [Met-(-1)]rOnc (SEQ ID NO:39) and $rEDN_{(1-21)}$rOnc were much less active with all of the substrates (non detectable or very little activity under the assay conditions employed). Surprisingly, the Gly containing hybrid protein, manifested significant ribonuclease activity especially under conditions optimal for EDN enzymatic activity. EDN is more active at a neutral pH (Sorrentino *et al.* (1992) *J. Biol. Chem* **267,** 14859-14865) and as seen in Table 2 there is a marked increase in EDN degradation of tRNA at pH 7.5 compared to pH 6 (42.3 fold). Also, behaving like EDN, the Gly-containing hybrid

increases in activity with a pH shift from 6 to 7.5 (21.7 fold) while nOnc loses activity at pH 7.5 consistent with its pH optimum that ranges from 6-6.5 (Ardelt *et al.* (1991) *J. Biol. Chem.* **256**, 245-251, Ardelt *et al.* (1994) *Protein Sci* **3**, **Suppl. 1**, 137). The enhanced EDN-like activity of the Gly-containing hybrid protein is also evidenced by its behavior with poly(A,C) which is an excellent substrate for EDN. As seen in Table 2, only rEDN$_{(1-21)}$rOncG26 expresses almost 50% of the enzymatic activity of EDN with this substrate whereas the activity of the other RNases are negligible. Similar results were observed with poly(U). In contrast, there was no detectable activity of rEDN or rEDN$_{(1-21)}$rOncG26 with UpG, an optimal Onconase substrate (Ardelt *et al.* (1994) *Protein Sci* **3**, **Suppl. 1**, 137). In summary, both [Met-(-1)]rOnc and rEDN$_{(1-21)}$rOnc are less enzymatically active than nOnc or rEDN. Although, rEDN$_{(1-21)}$rOncG26 expresses significant EDN-like enzymatic activity when assayed using defined substrates and conditions optimal for EDN, it is not as active as EDN in any assay. This could result from an impaired enzyme substrate interaction or from the use of suboptimal assay conditions for this hybrid enzyme.

Table 1 Activity of [Met-(-1)]rOnc or Hybrid Proteins in the Rabbit Reticulocyte Lysate compared to rEDN r nOnc in the Presence r Absence of PRI

	IC_{50}[a] (ng/ml)		
	(-)PRI	(+)PRI	Fold Difference
nOnc	0.2	0.24	1.2
rEDN	1.3	>40	>30.7
[Met-(-1)]rOnc	96	140	1.4
[Met-(-1)]rOncG20	28	24	0.9
$rEDN_{(1-21)}rOnc$	1600	3200	2
$rEDN_{(1-21)}rOncG26$	28	600	21

[a] IC_{50} is the concentration of protein necessary to inhibit protein synthesis by 50% in the rabbit reticulocyte lysate. Data points result from the average of at least three assays.

Table 2 Activity of RNases on Different Substrates

				RNase Activity	(units/mg protein)[a]	
Substrate	Assay pH	rEDN	nOnc	[Met-(-1)]rOnc	$rEND_{1-21r}Onc$	$rEDN_{1-21r}OncG_{26}$
Yeast RNA[a]	6.0	6000	560	0.01	8	120
tRNA[a,c]	6.0	1100	390	12	4	340
tRNA[a,c]	7.5	46000	60	50	130	7400
poly (A,C)[b]	7.0	8000	0.04	5	4.5	3900
UpG[b]	6.5	0.05	0.18	<0.01	<0.01	<0.01
poly U[b]	7.0	16.5	0.15	0.20	0.35	4.5

[a]RNase activity was quantitated through the formation of perchloric acid soluble nucleotides. Units are defined as the changes in A_{260} per minute calculated from the slopes of the linear part of the assays. Each value is the average of 2-3 assays in separate experiments.

[b]Spectrophotometric assays were performed according to Deprisco et al. (1984) and Libonati and Floridi (1969) as described in Materials and Methods. Units are defined as the changes in A_{260} per minute calculated from the slopes of the linear part of the assays. Each value is the average of two or more determinations.

[c][Met-(-1)]rOncG20 had no detectable activity.

F. Inhibition of protein synthesis in four human tumor cell lin s by RNases. The cytotoxic effect of [Met-(-1)]rOnc and the two hybrid RNases were compared to rEDN and nOnc by determining cell viability using the MTT assay. As depicted in Fig. 3, nOnc decreased tumor cell viability in all four human tumor cell lines. At the concentrations shown, rEDN had no effect on the viability of any of the cell lines. In contrast to nOnc, [Met-(-1)]rOnc as well as [Met-(-1)]rOncG20 was consistently less cytotoxic in all four cell lines. Yet, $rEDN_{(1-21)}$rOncG26 was more cytotoxic than nOnc in ACHN, human renal carcinoma cells and equally cytotoxic in the MDA-MB-231 human breast carcinoma cell line. Although $rEDN_{(1-21)}$rOncG26 was less active than nOnc in the SF-539 and HS 578T human glioma and breast cancer cell lines, respectively, it was still more active than [Met-(-1)]rOnc or $rEDN_{(1-21)}$rOnc protein containing Asn at position 26.

G. Structural Analysis of the hybrid RNases. Modeling the hybrid RNase was based on the alignment of the structures for Onc (Mosimann S.C., Ardelt W., James M.N.G., (1994), Refined 1.7 A X-ray crystallographic structure of P-30 protein, an amphibian ribonuclease with anti-tumor activity (*J Mol Biol* 236, 1141-1153) and EDN (Mosimann S.C., Newton D.L., Youle R.J., James M., X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83A resolution *J Mol Biol*). This and subsequent alignments were carried out using ALIGN (Satow Y., Cohen G.H., Padlan E.A., Davies D.R., (1986), *J. Mol Biol* 190, 593-604).

H. Modeling the structures of the hybrid RNases. The coordinates for Onc and EDN were superimposed on the basis of C^{α} trace alignment. Residues in conserved zones, particularly in the active site, showed very little displacement when comparing both structures (global r.m.s.d. of 1.44 A for 90 C^{α} atom pairs). The hybrid protein was modeled by manual rebuilding and geometry regularization using TOM (Cambillau C., Horjales E., (1987), *J. Mol Graph* 5, 174-177).

Subsequently, the models for $rEDN_{(1-21)}rOnc$ and $rEDN_{(1-21)}rOncG26$ were assigned an overall B-factor of 15 A^2 for all non-hydrogen atoms and independently subject to 300 cycles of positional energy minimization with the program XPLOR (Brunger A. (1992) XPLOR: a system for X-ray crystallography and NMR., New Haven: Yale University Press). The minimization yielded virtually identical structures in both cases, the highest distance based on C^α trace alignment being 0.44 for the C^α of the mutated residue 26. The geometry quality of the final models were assessed with PROCHECK (Laskowski R.A., MacArthur M.W., Moss D.S., Thornton J.M., (1993), *J Appl Crystallogr* 26, 283-291).

The structural basis for the marked differences in activity between the Gly and Asp containing hybrid RNases are not obvious from modeling these proteins especially since residue 26 is distant from the active site. When the highly homologous structure of RNase A complexed with a pentanucleotide (Fontecilla-Camps J.C., deLorens R., leDu M.H., Cuchillo C.M., (1994), *J. Biol Chem* 269, 21526-21531) was superimposed on the structure of the hybrid protein model, the nucleotide was observed also to be distant from the region of the mutation. However, the arrangement of the polynucleotide chain in the different RNases does not necessarily have to coincide. In the structure of EDN, a second sulfate ion was found in addition to the one in the active site (Mosimann S.C., Newton D.L., Youle R.J., James M., X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83A resolution *J Mol Biol*). This second sulfate is likely replacing a phosphate from the nucleotide to be cleaved, but no phosphate ion is located in the equivalent position in the RNase A-pentanucleotide complex. Moreover, one of the phosphates in this complex is forming a salt bridge with Lys-66, a residue which has no counterpart in Onc since it is located in a loop with a different topology in both molecules. Thus, whether the difference in enzymatic activity between the Asp and Gly mutants in the chimera is related to a change in the binding affinity for the substrate remains an open question.

Although the structural basis for the difference in the activities of the two EDN-Onc hybrids is not clear, the EDN-like behavior of the $rEDN_{(1-21)}$rOncG26 hybrid can likely be attributed to the configuration of the N-terminal region since both the pyroglutamic acid in nOnc and Lys-1 in EDN are located in the area of the active site (Mosimann S.C., Ardelt W., James M.N.G., (1994), Refined 1.7 A X-ray crystallographic structure of P-30 protein, an amphibian ribonuclease with anti-tumor activity *J Mol Biol* 236, 1141-1153; Mosimann S.C., Newton D.L., Youle R.J., James M., X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83A resolution *J Mol Biol*). In addition, the introduction of a Gly mutation in [Met-(-1)]rOnc did not significantly affect enzymatic activity. The preference of U over C in the B1 subsite of RNase A has been related to the presence of a particular residue (Asp-83) (DelCardayre S.B., Raines R.T., (1995), A residue to residue hydrogen bond mediates the nucleotide specificity of ribonuclease A *J Mol Biol* 252, 328-336). The corresponding residue in nOnc is also an aspartic acid (Asp-67), while in EDN this position is occupied by a histidine (His-82). EDN is more active toward poly (A,C), suggesting that it "prefers" C in the B1 subsite, possibly because it contains a histidine residue as opposed to the aspartic acid in nOnc and RNase A. Taken together, this could explain the decreased activity of the Gly containing hybrid relative to rEDN since, according to this hypothesis, the presence of the Asp residue contributed by the rOnc sequence would favor the binding of U over C. With regard to the difference in PRI inhibition, the superposition between the hybrid proteins and RNase A demonstrates that Asp-26 in the EDN-Onc chimeras is in the equivalent position to Asn-27 in RNase A that has been reported to be in contact with PRI (Kobe B., Deisenhofer J., (1995), *Nature* 374, 183-186). In addition, Asp-24 in both chimeras is very close to this region. Thus, the accumulation of negative charges in this area could prevent binding by the inhibitor. If so, the substitution of Gly for Asp would decrease the negative charge and restore the binding capacity.

Example II. rOnc-Antibody Fusion Proteins

Additional rOnc-antibody and ligand proteins have been produced and are highly active. E6FB[Met-(-1)]SerrOnc is an rOnc molecule having the nucleic acid sequence set out in SEQ ID NO:40 and the amino acid sequence set out in SEQ ID NO:41 and includes the Fv sequence from antibody E6, an anti-transferrin receptor antibody. See sequences for E6 at amino acid positions 1-237 in SEQ ID NO:41. "FB" refers to a linker used to link the antibody and the rOnc portion of the molecule and is found at nucleic acid positions 712 through 750 in SEQ ID NO:40. This molecule includes a Ser at amino acid position 252 instead of a Glu. E6FB[Met-(-1)]GlurOnc refers to the sequence in SEQ ID NO:40. Similar hybrid molecules have been made. The nucleic acid and amino acid sequences for Met-NLS(signal peptide)-Gln-rOncFBE6 are set out on SEQ ID NOS:42 and 43. Another E6/rOnc molecule is designated Met-Ser-rOncA87FBE6 and is found on SEQ ID NOS:44 and 45. "A87" refers to the fact that an Ala occurs at amino acid position 87.

Met-Ser-rOnc-Ang-FBE6 is set out on SEQ ID NOS:46 and 47.

E6FBMet-Ser-rOnc is set out on SEQ ID NOS:48 and 49.

Met-Glu-rOncFBE6 is set out on SEQ ID NOS:50 and 51.

Met-Ser-rOncFBE6 is set out on SEQ ID NOS:50 and 51, with the exception that Ser replaces Glu at amino acid position 2.

MOC31 and MOC162 refer to anti-colon cancer antibodies directed against the 17-1-A pancarcinoma antigen which were obtained from Dr. Hennie Hoogenboom. The Fv region of these antibodies was fused to rOnc. The nucleic acid and amino acid sequences for MetSerrOnc A87 FBMOC31 are set out on SEQ ID NOS:52 and 53. The nucleic acid and amino acid sequences for MOC31FBMetSerrOnc are set out on SEQ ID NOS:54 and 55. The nucleic acid and amino acid sequences for MetSerrOncFBMOC161 are set out on SEQ ID NOS:56 and 57.

The ligand, IL2 (interleukin 2) was recombinantly fused to rOnc as well. See SEQ ID NOS:58 and 59 for

IL2FBMetSerrOnc. See SEQ ID NOS:60 and 61 for MetSerrOncFBIL2.

Inhibition of protein synthesis in SF539 cells (which bear the transferrin receptor) was measured, as described above, for [Met-(-1)Ser]rOnc, E6FB[Met-(-1)Ser]rOnc; [Met-(-1)Ser]rOnc-AngFBE6 and [Met-(-1)Glu]rOncFBE6 constructs and compared with nOnc. The results are shown on Table 3. The three E6 constructs, in particular, had a very high level of activity -- up to 45 fold difference over the two non-E6 molecules. See also Figure 5. MetSerOncAng molecule was made corresponding to amino acids 1-107 of SEQ ID NO:47.

Table 3 Activity of modified rOnc and modified rOncFvs on protein synthesis

RNase	IC_{50} (nM)	Fold Difference
nOnc	10	1
[Met-(-1)Ser]rOnc	8	NSD
E6FB[Met-(-1)Ser]rOnc	0.22	45
[Met-(-1)Ser]rOnc-AngFBE6	0.27	37
[Met-(-1)Glu]rOncFBE6	0.50	20

The concentrations necessary to inhibit protein synthesis by 50% in SF539 human glioma cells. NSD, no significant difference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Rybak, Susanna M.
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Boque, Lluis
Wlodawer, Alexander

(ii) TITLE OF INVENTION: Recombinant Ribonuclease Proteins

(iii) NUMBER OF SEQUENCES: 39

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(F) ZIP: 94105-1492

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US Not yet assigned
(B) FILING DATE: Not yet assigned
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(B) TELEFAX: (415) 543-5043

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 104 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..104
(D) OTHER INFORMATION: /label= nOnc
/note= "native ONCONASE (Registered Trademark) from Rana pipiens"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /note= "Xaa = pyroglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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1               5                   10                  15

Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Ph  His Cys Lys Asp
            20                  25                  30

Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile Cys
        35                  40                  45

Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe Tyr
    50                  55                  60

Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu Lys
65                  70                  75                  80

Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro Val
                85                  90                  95

His Phe Val Gly Val Gly Ser Cys
            100
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..83
(D) OTHER INFORMATION: /note= "Rana clone 9 peptide from Rana pipiens genomic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1               5                   10                  15

Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
            20                  25                  30

Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
        35                  40                  45

Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
    50                  55                  60

Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
65                  70                  75                  80

Val His Phe
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /note= "N-terminal sequence of nOnc with Glu in place of pyroglutamic acid in position 1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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1               5                   10                  15

Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..34
(D) OTHER INFORMATION: /note= "N-terminal sequence of recombinant eosinophil-derived neurotoxin (rEDN)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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1               5                   10                  15

Asn Met Thr Ser Gln Gln Cys Thr Asn Ala Met Gln Val Ile Asn Asn
            20                  25                  30

Tyr Gln
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /note= "N-terminal sequence of [Met-(-1)]rOncG20, containing a Gly to Asp substitution at position 20 of [Met-(-1)]rOnc, and without the extra N-terminal Met from the E. coli bacterial expression system"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Glu Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg Asp
1               5                   10                  15

Val Asp Cys Gly Asn Ile Met Ser Thr Asn Leu Phe
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..34
(D) OTHER INFORMATION: /note= "N-terminal sequence of rEDN1-21rOnc, without the extra N-terminal Met from the E. coli bacterial expression system"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe Glu Thr Gln His Ile
1               5                   10                  15

Asn Met Thr Ser Gln Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn
            20                  25                  30

Leu Phe
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..34
(D) OTHER INFORMATION: /not = "N-terminal sequence of rEDN1-21rOncG26, containing a Gly to Asp substitution at position 26 of rEDN1-21rOnc, and without the extra N-terminal Met from the E. coli bacterial expression system"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe Glu Thr Gln His Ile
1               5                   10                  15

Asn Met Thr Ser Gln Asp Val Asp Cys Gly Asn Ile Met Ser Thr Asn
            20                  25                  30

Leu Phe
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..111
(D) OTHER INFORMATION: /note= "Frog Lectin from Rana catesbeiana"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Glu Asn Trp Ala Thr Phe Gln Gln Lys His Ile Ile Asn Thr Pro Ile
1               5                   10                  15

Ile Asn Cys Asn Thr Ile Met Asp Asn Asn Ile Tyr Ile Val Gly Gly
            20                  25                  30

Gln Cys Lys Arg Val Asn Thr Phe Ile Ile Ser Ser Ala Thr Thr Val
        35                  40                  45

Lys Ala Ile Cys Thr Gly Val Ile Asn Met Asn Val Leu Ser Thr Thr
    50                  55                  60

Arg Phe Gln Leu Asn Thr Cys Thr Arg Thr Ser Ile Thr Pro Arg Pro
65                  70                  75                  80

Cys Pro Tyr Ser Ser Arg Thr Glu Thr Asn Tyr Ile Cys Val Lys Cys
                85                  90                  95

Glu Asn Gln Tyr Pro Val His Phe Ala Gly Ile Gly Arg Cys Pro
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..134
(D) OTHER INFORMATION: /note= "Human eosinophil-derived neurotoxin (EDN)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe Glu Thr Gln His Ile
1               5                   10                  15

Asn Met Thr Ser Gln Gln Cys Thr Asn Ala Met Gln Val Ile Asn Asn
            20                  25                  30

Tyr Gln Arg Arg Cys Lys Asn Gln Asn Thr Phe Leu Leu Thr Thr Phe
        35                  40                  45

Ala Asn Val Val Asn Val Cys Gly Asn Pro Asn Met Thr Cys Pro Ser
    50                  55                  60

Asn Lys Thr Arg Lys Asn Cys His His Ser Gly Ser Gln Val Pro Leu
65                  70                  75                  80

Ile His Cys Asn Leu Thr Thr Pro Ser Pro Gln Asn Ile Ser Asn Cys
                85                  90                  95

Arg Tyr Ala Gln Thr Pro Ala Asn Met Phe Tyr Ile Val Ala Cys Asp
            100                 105                 110

Asn Arg Asp Gln Arg Arg Asp Pro Pro Gln Tyr Pro Val Val Pro Val
        115                 120                 125

His Leu Asp Arg Ile Ile
    130
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..133
(D) OTHER INFORMATION: /note= "Human eosinophil cationic protein (ECP)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Arg Pro Pro Gln Phe Thr Arg Ala Gln Trp Phe Ala Ile Gln His Ile
1               5                   10                  15

Ser Leu Asn Pro Pro Arg Cys Thr Ile Ala Met Arg Ala Ile Asn Asn
            20                  25                  30

Tyr Arg Trp Arg Cys Lys Asn Gln Asn Thr Phe Leu Arg Thr Thr Phe
        35                  40                  45

Ala Asn Val Val Asn Val Cys Gly Asn Gln Ser Ile Arg Cys Pro His
    50                  55                  60

Asn Arg Thr Leu Asn Asn Cys His Arg Ser Arg Phe Arg Val Pro Leu
65                  70                  75                  80

Leu His Cys Asp Leu Ile Asn Pro Gly Ala Gln Asn Ile Ser Asn Cys
                85                  90                  95

Arg Tyr Ala Asp Arg Pro Gly Arg Arg Phe Tyr Val Val Ala Cys Asp
            100                 105                 110

Asn Arg Asp Pro Arg Asp Ser Pro Arg Tyr Pro Val Val Pro Val His
        115                 120                 125

Leu Asp Thr Thr Ile
    130
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..125
(D) OTHER INFORMATION: /note= "Bovine angiogenin (Ang)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Ala Gln Asp Asp Tyr Arg Tyr Ile His Phe Leu Thr Gln His Tyr Asp
1               5                   10                  15

Ala Lys Pro Lys Gly Arg Asn Asp Glu Tyr Cys Phe His Met Met Lys
            20                  25                  30

Asn Arg Arg Leu Thr Arg Pro Cys Lys Asp Arg Asn Thr Phe Ile His
        35                  40                  45

Gly Asn Lys Asn Asp Ile Lys Ala Ile Cys Glu Asp Arg Asn Gly Gln
    50                  55                  60

Pro Tyr Arg Gly Asp Leu Arg Ile Ser Lys Ser Glu Phe Gln Ile Thr
65                  70                  75                  80

Ile Cys Lys His Lys Gly Gly Ser Ser Arg Pro Pro Cys Arg Tyr Gly
                85                  90                  95
```

Ala Thr Glu Asp Ser Arg Val Ile Val Val Gly Cys Glu Asn Gly Leu
100 105 110

Pro Val His Phe Asp Glu Ser Phe Ile Thr Pro Arg His
115 120 125

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..124
(D) OTHER INFORMATION: /note= "Bovine seminal RNase"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Lys Glu Ser Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Gly
1 5 10 15

Asn Ser Pro Ser Ser Ser Ser Asn Tyr Cys Asn Leu Met Met Cys Cys
20 25 30

Arg Lys Met Thr Gln Gly Lys Cys Lys Pro Val Asn Thr Phe Val His
35 40 45

Glu Ser Leu Ala Asp Val Lys Ala Val Cys Ser Gln Lys Lys Val Thr
50 55 60

Cys Lys Asn Gly Gln Thr Asn Cys Tyr Gln Ser Lys Ser Thr Met Arg
65 70 75 80

Ile Thr Asp Cys Arg Glu Thr Gly Ser Ser Lys Tyr Pro Asn Cys Ala
85 90 95

Tyr Lys Thr Thr Gln Val Glu Lys His Ile Ile Val Ala Cys Gly Gly
100 105 110

Lys Pro Ser Val Pro Val His Phe Asp Ala Ser Val
115 120

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..124
(D) OTHER INFORMATION: /note= "Bovine pancreatic RNase A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Ser
1               5                   10                  15

Thr Ser Ala Ala Ser Ser Ser Asn Tyr Cys Asn Gln Met Met Lys Ser
            20                  25                  30

Arg Asn Leu Thr Lys Asp Arg Cys Lys Pro Val Asn Thr Phe Val His
        35                  40                  45

Glu Ser Leu Ala Asp Val Gln Ala Val Cys Ser Gln Lys Asn Val Ala
    50                  55                  60

Cys Lys Asn Gly Gln Thr Asn Cys Tyr Gln Ser Tyr Ser Thr Met Ser
65                  70                  75                  80

Ile Thr Asp Cys Arg Glu Thr Gly Ser Ser Lys Tyr Pro Asn Cys Ala
                85                  90                  95

Tyr Lys Thr Thr Gln Ala Asn Lys His Ile Ile Val Ala Cys Glu Gly
            100                 105                 110

Asn Pro Val Val Pro Val His Phe Asp Ala Ser Val
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Xaa = Ser, Tyr or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Lys Xaa Pro
1
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa = Ser, Tyr or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Lys Pro Xaa
1

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Xaa = Ser, Tyr or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Asn Xaa Pro
1

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa = Ser, Tyr or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Xaa Lys Pro
1

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa = Ser, Tyr or Thr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Xaa Pro Lys
1
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 321 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..321
(D) OTHER INFORMATION: /note= "MetSerOnc86Ang104"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
ATC TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG      48
Ile Ser Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
  1               5                  10                  15

GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG      96
Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
             20                  25                  30

GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC     144
Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
         35                  40                  45

TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG TTT     192
Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
     50                  55                  60

TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA     240
Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
 65                  70                  75                  80

AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA     288
Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
                 85                  90                  95

GTT CAT TTT GTT CAG TCA ATT TTC CGT CGT CCG                         321
Val His Phe Val Gln Ser Ile Phe Arg Arg Pro
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Il  Ser Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
 1              5                   10                  15

Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
            20                  25                  30

Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
        35                  40                  45

Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
    50                  55                  60

Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
65                  70                  75                  80

Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
                85                  90                  95

Val His Phe Val Gln Ser Ile Phe Arg Arg Pro
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..333
(D) OTHER INFORMATION: /note= "EDNGlyOnc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
ATG AAA CCG CCG CAG TTC ACT TGG GCT CAG TGG TTC GAA ACT CAG CAT        48
Met Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe Glu Thr Gln His
 1              5                   10                  15

ATC AAC ATG ACT TCT CAG GAT GTT GAT TGT GGT AAT ATC ATG TCA ACA        96
Ile Asn Met Thr Ser Gln Asp Val Asp Cys Gly Asn Ile Met Ser Thr
            20                  25                  30

AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT       144
Asn Leu Phe His Cys Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro
        35                  40                  45

GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG       192
Glu Pro Val Lys Ala Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn Val
    50                  55                  60

TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG       240
Leu Thr Thr Ser Glu Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg
65                  70                  75                  80

CCT TGC AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT       288
Pro Cys Lys Tyr Lys Leu Lys Lys Ser Thr Asn Lys Phe Cys Val Thr
                85                  90                  95
```

```
TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT      333
Cys Glu Asn Gln Ala Pro Val His Phe Val Gly Val Gly Ser Cys
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Met Lys Pro Pro Gln Phe Thr Trp Ala Gln Trp Phe Glu Thr Gln His
1               5                   10                  15

Ile Asn Met Thr Ser Gln Asp Val Asp Cys Gly Asn Ile Met Ser Thr
            20                  25                  30

Asn Leu Phe His Cys Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro
        35                  40                  45

Glu Pro Val Lys Ala Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn Val
    50                  55                  60

Leu Thr Thr Ser Glu Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg
65                  70                  75                  80

Pro Cys Lys Tyr Lys Leu Lys Lys Ser Thr Asn Lys Phe Cys Val Thr
                85                  90                  95

Cys Glu Asn Gln Ala Pro Val His Phe Val Gly Val Gly Ser Cys
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..315
(D) OTHER INFORMATION: /note= "MetTyrrOnc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
ATG TAT GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG      48
Met Tyr Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
1               5                   10                  15

GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG      96
Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
            20                  25                  30
```

```
GAC AAG AAC ACT TTT ACT TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC      144
Asp Lys Asn Thr Phe Thr Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
         35                  40                  45

TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG TTT      192
Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
     50                  55                  60

TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA      240
Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
 65                  70                  75                  80

AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA      288
Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
                 85                  90                  95

GTT CAT TTT GTT GGA GTT GGA TCT TGT                                  315
Val His Phe Val Gly Val Gly Ser Cys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Met Tyr Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
 1               5                  10                  15

Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
            20                  25                  30

Asp Lys Asn Thr Phe Thr Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
         35                  40                  45

Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
     50                  55                  60

Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
 65                  70                  75                  80

Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
                 85                  90                  95

Val His Phe Val Gly Val Gly Ser Cys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..315
(D) OTHER INFORMATION: /note= "MetSerrOnc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG        48
Met Ser Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
  1               5                  10                  15

GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG        96
Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
             20                  25                  30

GAC AAG AAC ACT TTT ACT TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC       144
Asp Lys Asn Thr Phe Thr Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
         35                  40                  45

TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG TTT       192
Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
     50                  55                  60

TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA       240
Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
 65                  70                  75                  80

AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA       288
Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
                 85                  90                  95

GTT CAT TTT GTT GGA GTT GGA TCT TGT                                   315
Val His Phe Val Gly Val Gly Ser Cys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Ser Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
  1               5                  10                  15

Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
             20                  25                  30

Asp Lys Asn Thr Phe Thr Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
         35                  40                  45

Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
     50                  55                  60

Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
 65                  70                  75                  80

Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
                 85                  90                  95
```

```
Val His Phe Val Gly Val Gly Ser Cys
        100                 105

(2) INFORMATION FOR SEQ ID NO:27:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 318 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 1..318
          (D) OTHER INFORMATION: /note= "MetLysTyrrOnc"

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATG AAA TAT GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA      48
Met Lys Tyr Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr
 1               5                  10                  15

AGG GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC      96
Arg Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys
            20                  25                  30

AAG GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC     144
Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala
        35                  40                  45

ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG     192
Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu
    50                  55                  60

TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA     240
Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys
 65                  70                  75                  80

TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA     288
Leu Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala
                85                  90                  95

CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT                             318
Pro Val His Phe Val Gly Val Gly Ser Cys
            100                 105

(2) INFORMATION FOR SEQ ID NO:28:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 106 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Lys Tyr Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr
 1               5                  10                  15

Arg Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys
            20                  25                  30
```

Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala
35 40 45

Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu
50 55 60

Ph Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys
65 70 75 80

Leu Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala
85 90 95

Pro Val His Phe Val Gly Val Gly Ser Cys
100 105

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 321 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..321
(D) OTHER INFORMATION: /note= "MetAlaAlaTyrrOnc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ATG GCT GCT TAT GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC 48
Met Ala Ala Tyr Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn
1 5 10 15

ACA AGG GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC 96
Thr Arg Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His
20 25 30

TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG 144
Cys Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys
35 40 45

GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT 192
Ala Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser
50 55 60

GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT 240
Glu Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr
65 70 75 80

AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG 288
Lys Leu Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln
85 90 95

GCA CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT 321
Ala Pro Val His Phe Val Gly Val Gly Ser Cys
100 105

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Met Ala Ala Tyr Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn
 1               5                  10                  15

Thr Arg Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His
            20                  25                  30

Cys Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys
        35                  40                  45

Ala Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser
    50                  55                  60

Glu Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr
65                  70                  75                  80

Lys Leu Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln
                85                  90                  95

Ala Pro Val His Phe Val Gly Val Gly Ser Cys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 336 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..336
(D) OTHER INFORMATION: /note= "NLSMetSerrOnc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
CCC AAG AAG AAG CGG AAG GTG ATG TCA GAT TGG CTT ACA TTT CAG AAA      48
Pro Lys Lys Lys Arg Lys Val Met Ser Asp Trp Leu Thr Phe Gln Lys
 1               5                  10                  15

AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT GAT AAT ATC ATG TCA      96
Lys His Ile Thr Asn Thr Arg Asp Val Asp Cys Asp Asn Ile Met Ser
            20                  25                  30

ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA CGT     144
Thr Asn Leu Phe His Cys Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg
        35                  40                  45

CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT     192
Pro Glu Pro Val Lys Ala Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn
    50                  55                  60
```

```
GTG TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC      240
Val Leu Thr Thr Ser Glu Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser
 65                  70                  75                  80

AGG CCT TGC AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA      288
Arg Pro Cys Lys Tyr Lys Leu Lys Lys Ser Thr Asn Lys Phe Cys Val
                 85                  90                  95

ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT      336
Thr Cys Glu Asn Gln Ala Pro Val His Phe Val Gly Val Gly Ser Cys
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Pro Lys Lys Lys Arg Lys Val Met Ser Asp Trp Leu Thr Phe Gln Lys
 1               5                  10                  15

Lys His Ile Thr Asn Thr Arg Asp Val Asp Cys Asp Asn Ile Met Ser
            20                  25                  30

Thr Asn Leu Phe His Cys Lys Asp Lys Asn Thr Phe Ile Tyr Ser Arg
        35                  40                  45

Pro Glu Pro Val Lys Ala Ile Cys Lys Gly Ile Ile Ala Ser Lys Asn
    50                  55                  60

Val Leu Thr Thr Ser Glu Phe Tyr Leu Ser Asp Cys Asn Val Thr Ser
 65                  70                  75                  80

Arg Pro Cys Lys Tyr Lys Leu Lys Lys Ser Thr Asn Lys Phe Cys Val
                 85                  90                  95

Thr Cys Glu Asn Gln Ala Pro Val His Phe Val Gly Val Gly Ser Cys
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa = an aliphatic amino acid, Ala, Leu, Ile, Val, or Pro"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /note= "Xaa = an aliphatic amino acid, Ala, Leu, Ile, Val or Pro"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa = Ser, Met, Cys, Ala or Gln"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Xaa Xaa Xaa
1

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Cys Val Ile Met
1

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AGRGATGTKG ATTGYGATAA YATCATG 27

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGTGARAAYC AGGCMCCWGT KCAYTTT 27

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 249 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION: 1..249
(D) OTHER INFORMATION: /note= "Rana 9"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
GATGTTGATT GTGATAATAT CATGTCAACA AACTTGTTCC ACTGCAAGGA CAAGAACACT     60

TTTATCTATT CACGTCCTGA GCCAGTGAAG GCCATCTGTA AAGGAATTAT AGCCTCCAAA    120

AATGTGTTAA CTACCTCTGA GTTTTATCTC TCTGATTGCA ATGTAACAAG CAGGCCTTGC    180

AAGTATAAAT TAAAGAAATC AACTAATAAA TTTTGTGTAA CTTGTGAAAA TCAGGCACCA    240

GTTCATTTT                                                            249
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 315 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..315
(D) OTHER INFORMATION: /note= "[Met-(-1)]rOnc"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
ATG GAG GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG       48
Met Glu Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
  1               5                  10                  15

GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG       96
Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
             20                  25                  30

GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC      144
Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
         35                  40                  45

TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG TTT      192
Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
     50                  55                  60
```

TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA 240
Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
65 70 75 80

AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA 288
Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
85 90 95

GTT CAT TTT GTT GGA GTT GGA TCT TGT 315
Val His Phe Val Gly Val Gly Ser Cys
100 105

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Glu Asp Trp Leu Thr Phe Gln Lys Lys His Ile Thr Asn Thr Arg
1 5 10 15

Asp Val Asp Cys Asp Asn Ile Met Ser Thr Asn Leu Phe His Cys Lys
20 25 30

Asp Lys Asn Thr Phe Ile Tyr Ser Arg Pro Glu Pro Val Lys Ala Ile
35 40 45

Cys Lys Gly Ile Ile Ala Ser Lys Asn Val Leu Thr Thr Ser Glu Phe
50 55 60

Tyr Leu Ser Asp Cys Asn Val Thr Ser Arg Pro Cys Lys Tyr Lys Leu
65 70 75 80

Lys Lys Ser Thr Asn Lys Phe Cys Val Thr Cys Glu Asn Gln Ala Pro
85 90 95

Val His Phe Val Gly Val Gly Ser Cys
100 105

sFvFBmetgluOnc -> 1-phase Translation

```
DNA  sequence    1065 b.p.   GACATCAAGATG ... GTTGGATCTTGT   linear
|---->  EGSFV
1   /   1                                   31   /   11
GAC ATC AAG ATG ACC CAG TCT CCA TCT TCC ATG TAT GCA TCT CTA GGA GAG AGA GTC ACT
asp ile lys met thr gln ser pro ser ser met tyr ala ser leu gly glu arg val thr
61  /   21                                  91   /   31
TTC ACT TGC AAG GCG AGT CAG GAC ATT AAT AAC TAT TTA TGC TGG TTC CAG CAG AAA CCA
phe thr cys lys ala ser gln asp ile asn asn tyr leu cys trp phe gln gln lys pro
121 /   41                                  151  /   51
GGG AAA TCT CCT AAG ACC CTG ATC TAT CGT GCA AAC AGA CTG GTA GAT GGG GTC CCA TCA
gly lys ser pro lys thr leu ile tyr arg ala asn arg leu val asp gly val pro ser
181 /   61                                  211  /   71
AGG TTC AGT GGC AGT GGA TCT GGA CAA GAT TAT TCT CTC ACC ATT AGC AGC CTG GAG TAT
arg phe ser gly ser gly ser gly gln asp tyr ser leu thr ile ser ser leu glu tyr     EGSFV
241 /   81                                  271  /   91
GAA GAT ATG GGA ATT TAT TAT TGT CTA CAG TAT GAT GAG TTT CCG TAC ACG TTC GGA GGG
glu asp met gly ile tyr tyr cys leu gln tyr asp glu phe pro tyr thr phe gly gly
301 /   101                                 331  /   111
GGG ACC AAG CTG GAA ATA AAA GGA GGC GGT GGC TCG GGC GGT GGC GGA TCG GGT GGC GGC
gly thr lys leu glu ile lys gly gly gly gly ser gly gly gly gly ser gly gly gly
361 /   121                                 391  /   131
GGC TCT GAG GTT CAG CTC CAG CAG TCT GGG ACT GTA CTG GCA AGG CCT GGG GCT TCA GTG
gly ser glu val gln leu gln gln ser gly thr val leu ala arg pro gly ala ser val
421 /   141                                 451  /   151
AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT TCC AGC TAC TGG ATG CAC TGG ATA AAA
lys met ser cys lys ala ser gly tyr thr phe ser ser tyr trp met his trp ile lys
481 /   161                                 511  /   171
CAG AGG CCT GGA CAG GGT CTG GAC TGG ATT GTC GCT ATT GAT CCT CGA AAT AGT GAT ACT
gln arg pro gly gln gly leu asp trp ile val ala ile asp pro arg asn ser asp thr
541 /   181                                 571  /   191
ATT TAC AAC CCG CAA TTC AAA CAC AAG GCC AAA CTG ACT GCA GTC ACC TCC ACC AGC ACT
ile tyr asn pro gln phe lys his lys ala lys leu thr ala val thr ser thr ser thr
601 /   201                                 631  /   211
GCC TAC ATG GAA CTC AAC AGC CTG ACA AAT GAG GAC TCT GCG GTC TAT TAC TGT ACC CCT
ala tyr met glu leu asn ser leu thr asn glu asp ser ala val tyr tyr cys thr pro
661 /   221                                 691  /   231           <--|--> FB
CTT TAT TAC TTT GAC TCC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAG AAA
leu tyr tyr phe asp ser trp gly gln gly thr thr leu thr val ser ser ala lys lys
721 /   241                          <-- 751  /   251 --> met glu onc
CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT ATG GAG GAT TGG CTT ACA TTT CAG AAA AAA
leu asn asp ala gln ala pro lys ser asp met glu asp trp leu thr phe gln lys lys     met glu onc
781 /   261                                 811 ser 271
CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC
his ile thr asn thr arg asp val asp cys asp asn ile met ser thr asn leu phe his
841 /   281                                 871  /   291
TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA
cys lys asp lys asn thr phe ile tyr ser arg pro glu pro val lys ala ile cys lys
901 /   301                                 931  /   311
GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT
gly ile ile ala ser lys asn val leu thr thr ser glu phe tyr leu ser asp cys asn
961 /   321                                 991  /   331
GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT
val thr ser arg pro cys lys tyr lys leu lys lys ser thr asn lys phe cys val thr
1021 /  341                                 1051 /   351
TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT
cys glu asn gln ala pro val his phe val gly val gly ser cys
```

SEQ ID NOS:40,41

SigpepglnOncFBE6 -> 1-phase Translation

DNA sequence 1137 b.p. ATGGGTCTGGAA ... ACAGTCTCCTCA linear

Signal peptide

```
1   /   1                                   31  /  11
ATG GGT CTG GAA AAA TCT CTG ATC CTG TTC CCG CTG TTC TTC CTG CTG CTG GGT TGG GTT
met gly leu glu lys ser leu ile leu phe pro leu phe phe leu leu leu gly trp val
61  /  21        gln onc                    91  /  31
CAG CCG TCT CTG GGT CAG GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG
gln pro ser leu gly gln asp trp leu thr phe gln lys lys his ile thr asn thr arg
121 /  41                                   151 /  51
GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT
asp val asp cys asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr
181 /  61                                   211 /  71
TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA
ph  ile tyr ser arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys
241 /  81                                   271 /  91
AAT GTG TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC
asn val leu thr thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys
301 / 101                                   331 / 111
AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA
lys tyr lys leu lys lys ser thr asn lys phe cys val thr cys glu asn gln ala pro
361 / 121                                   391 / 131   FB
GTT CAT TTT GTT GGA GTT GGA TCT TGT GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG
val his phe val gly val gly ser cys ala lys lys leu asn asp ala gln ala pro lys
421   141   E6 scFv                         451 / 151
AGT GAT GAC ATC AAG ATG ACC CAG TCT CCA TCT TCC ATG TAT GCA TCT CTA GGA GAG AGA
ser asp asp ile lys met thr gln ser pro ser ser met tyr ala ser leu gly glu arg
481 / 161                                   511 / 171
GTC ACT TTC ACT TGC AAG GCG AGT CAG GAC ATT AAT AAC TAT TTA TGC TGG TTC CAG CAG
val thr phe thr cys lys ala ser gln asp ile asn asn tyr leu cys trp phe gln gln
541 / 181                                   571 / 191
AAA CCA GGG AAA TCT CCT AAG ACC CTG ATC TAT CGT GCA AAC AGA CTG GTA GAT GGG GTC
lys pro gly lys ser pro lys thr leu ile tyr arg ala asn arg leu val asp gly val
601 / 201                                   631 / 211
CCA TCA AGG TTC AGT GGC AGT GGA TCT GGA CAA GAT TAT TCT CTC ACC ATT AGC AGC CTG
pro ser arg phe ser gly ser gly ser gly gln asp tyr ser leu thr ile ser ser leu
661 / 221                                   691 / 231
GAG TAT GAA GAT ATG GGA ATT TAT TAT TGT CTA CAG TAT GAT GAG TTT CCG TAC ACG TTC
glu tyr glu asp met gly ile tyr tyr cys leu gln tyr asp glu phe pro tyr thr phe
721 / 241                                   751 / 251
GGA GGG GGG ACC AAG CTG GAA ATA AAA GGA GGC GGT GGC TCG GGC GGT GGC GGA TCG GGT
gly gly gly thr lys leu glu ile lys gly gly gly gly ser gly gly gly gly ser gly
781 / 261                                   811 / 271
GGC GGC GGC TCT GAG GTT CAG CTC CAG CAG TCT GGG ACT GTA CTG GCA AGG CCT GGG GCT
gly gly gly ser glu val gln leu gln gln ser gly thr val leu ala arg pro gly ala
841 / 281                                   871 / 291
TCA GTG AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT TCC AGC TAC TGG ATG CAC TGG
ser val lys met ser cys lys ala ser gly tyr thr phe ser ser tyr trp met his trp
901 / 301                                   931 / 311
ATA AAA CAG AGG CCT GGA CAG GGT CTG GAC TGG ATT GTC GCT ATT GAT CCT CGA AAT AGT
ile lys gln arg pro gly gln gly leu asp trp ile val ala ile asp pro arg asn ser
961 / 321                                   991 / 331
GAT ACT ATT TAC AAC CCG CAA TTC AAA CAC AAG GCC AAA CTG ACT GCA GTC ACC TCC ACC
asp thr ile tyr asn pro gln phe lys his lys ala lys leu thr ala val thr ser thr
1021 / 341                                  1051 / 351
AGC ACT GCC TAC ATG GAA CTC AAC AGC CTG ACA AAT GAG GAC TCT GCG GTC TAT TAC TGT
ser thr ala tyr met glu leu asn ser leu thr asn glu asp ser ala val tyr tyr cys
1081 / 361                                  1111 / 371
ACC CCT CTT TAT TAC TTT GAC TCC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA
thr pro leu tyr tyr phe asp ser trp gly gln gly thr thr leu thr val ser ser
```

gln onc

E6 scFv

SEQ ID NOS:42,43

```
MetserOncA87FBE6 -> 1-phase Translation

DNA sequence   1074 b.p.   ATGTCAGATTGG ... TCACATCACCAT   linear
                Met Ser Onc A87
1   /  .1                                       31  /  11
ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT
met ser asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp cys
61  /  21                                       91  /  31
GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA      Met Ser Onc AS:
asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr ser
121 /  41                                       151 /  51
CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT
arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu thr
181 /  61                                       211 /  71
ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA
thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys leu
241 /  81                                       271 /  91
AAG AAA TCA ACT AAT AAA TTT GCT GTA ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT
lys lys ser thr asn lys phe ala val thr cys glu asn gln ala pro val his phe val
301 /  101                                      331 /  111    F8                  E6 sFv
GGA GTT GGA TCT TGT GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT GAC ATC      FB
gly val gly ser cys ala lys lys leu asn asp ala gln ala pro lys ser asp asp ile
361 /  121                                      391 /  131
AAG ATG ACC CAG TCT CCA TCT TCC ATG TAT GCA TCT CTA GGA GAG AGA GTC ACT TTC ACT
lys met thr gln ser pro ser ser met tyr ala ser leu gly glu arg val thr phe thr
421 /  141                                      451 /  151
TGC AAG GCG AGT CAG GAC ATT AAT AAC TAT TTA TGC TGG TTC CAG CAG AAA CCA GGG AAA
cys lys ala ser gln asp ile asn asn tyr leu cys trp phe gln gln lys pro gly lys
481 /  161                                      511 /  171
TCT CCT AAG ACC CTG ATC TAT CGT GCA AAC AGA CTG GTA GAT GGG GTC CCA TCA AGG TTC
ser pro lys thr leu ile tyr arg ala asn arg leu val asp gly val pro ser arg phe
541 /  181                                      571 /  191
AGT GGC AGT GGA TCT GGA CAA GAT TAT TCT CTC ACC ATT AGC AGC CTG GAG TAT GAA GAT
ser gly ser gly ser gly gln asp tyr ser leu thr ile ser ser leu glu tyr glu asp
601 /  201                                      631 /  211
ATG GGA ATT TAT TAT TGT CTA CAG TAT GAT GAG TTT CCG TAC ACG TTC GGA GGG GGG ACC
met gly ile tyr tyr cys leu gln tyr asp glu phe pro tyr thr phe gly gly gly thr
661 /  221                                      691 /  231
AAG CTG GAA ATA AAA GGA GGC GGT GGC TCG GGC GGT GGC GGA TCG GGT GGC GGC GGC TCT      E6 sFv
lys leu glu ile lys gly gly gly gly ser gly gly gly gly ser gly gly gly gly ser
721 /  241                                      751 /  251
GAG GTT CAG CTC CAG CAG TCT GGG ACT GTA CTG GCA AGG CCT GGG GCT TCA GTG AAG ATG
glu val gln leu gln gln ser gly thr val leu ala arg pro gly ala ser val lys met
781 /  261                                      811 /  271
TCC TGC AAG GCT TCT GGC TAC ACC TTT TCC AGC TAC TGG ATG CAC TGG ATA AAA CAG AGG
ser cys lys ala ser gly tyr thr phe ser ser tyr trp met his trp ile lys gln arg
841 /  281                                      871 /  291
CCT GGA CAG GGT CTG GAC TGG ATT GTC GCT ATT GAT CCT CGA AAT AGT GAT ACT ATT TAC
pro gly gln gly leu asp trp ile val ala ile asp pro arg asn ser asp thr ile tyr
901 /  301                                      931 /  311
AAC CCG CAA TTC AAA CAC AAG GCC AAA CTG ACT GCA GTC ACC TCC ACC AGC ACT GCC TAC
asn pro gln phe lys his lys ala lys leu thr ala val thr ser thr ser thr ala tyr
961 /  321                                      991 /  331
ATG GAA CTC AAC AGC CTG ACA AAT GAG GAC TCT GCG GTC TAT TAC TGT ACC CCT CTT TAT
met glu leu asn ser leu thr asn glu asp ser ala val tyr tyr cys thr pro leu tyr
1021 / 341          E6 sFv                      1051 / 351
TAC TTT GAC TCC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA CAT CAC CAT
tyr phe asp ser trp gly gln gly thr thr leu thr val ser ser his his his
```

SEQ ID NOS:44,45

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MET SER CMC-ANGsFv -> 1-phase Translation

DNA sequence   1086 b.p.   ATGTCAGATTGG ... CACCATTAGTAG   linear
         met ser onc-Ang
1    /   1                                31   /   11
ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT
met ser asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp cys
61   /   21                               91   /   31
GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA
asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr ser
121  /   41                               151  /   51
CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT
arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu thr
181  /   61                               211  /   71
ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA
thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys leu
241  /   81                               271  /   91
AAG AAA TCA ACT AAT AAA TTT GTT GTT GCT TGT GAA AAT GGC TTA CCT GTC CAC TTG GAT
lys lys ser thr asn lys phe val val ala cys glu asn gly leu pro val his leu asp
301  /   101                 FB           331  /   111
CAG TCA ATT TTC CGT CGT CCG GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT
gln ser ile phe arg arg pro ala lys lys leu asn asp ala gln ala pro lys ser asp
361  /   121 GbsFv                        391  /   131
GAC ATC AAG ATG ACC CAG TCT CCA TCT TCC ATG TAT GCA TCT CTA GGA GAG AGA GTC ACT
asp ile lys met thr gln ser pro ser ser met tyr ala ser leu gly glu arg val thr
421  /   141                              451  /   151
TTC ACT TGC AAG GCG AGT CAG GAC ATT AAT AAC TAT TTA TGC TGG TTC CAG CAG AAA CCA
phe thr cys lys ala ser gln asp ile asn asn tyr leu cys trp phe gln gln lys pro
481  /   161                              511  /   171
GGG AAA TCT CCT AAG ACC CTG ATC TAT CGT GCA AAC AGA CTG GTA GAT GGG GTC CCA TCA
gly lys ser pro lys thr leu ile tyr arg ala asn arg leu val asp gly val pro ser
541  /   181                              571  /   191
AGG TTC AGT GGC AGT GGA TCT GGA CAA GAT TAT TCT CTC ACC ATT AGC AGC CTG GAG TAT
arg phe ser gly ser gly ser gly gln asp tyr ser leu thr ile ser ser leu glu tyr
601  /   201                              631  /   211
GAA GAT ATG GGA ATT TAT TAT TGT CTA CAG TAT GAT GAG TTT CCG TAC ACG TTC GGA GGG
glu asp met gly ile tyr tyr cys leu gln tyr asp glu phe pro tyr thr phe gly gly
661  /   221                              691  /   231
GGG ACC AAG CTG GAA ATA AAA GGA GGC GGT GGC TCG GGC GGT GGC GGA TCG GGT GGC GGC
gly thr lys leu glu ile lys gly gly gly gly ser gly gly gly gly ser gly gly gly
721  /   241                              751  /   251
GGC TCT GAG GTT CAG CTC CAG CAG TCT GGG ACT GTA CTG GCA AGG CCT GGG GCT TCA GTG
gly ser glu val gln leu gln gln ser gly thr val leu ala arg pro gly ala ser val
781  /   261                              811  /   271
AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT TCC AGC TAC TGG ATG CAC TGG ATA AAA
lys met ser cys lys ala ser gly tyr thr phe ser ser tyr trp met his trp ile lys
841  /   281                              871  /   291
CAG AGG CCT GGA CAG GGT CTG GAC TGG ATT GTC GCT ATT GAT CCT CGA AAT AGT GAT ACT
gln arg pro gly gln gly leu asp trp ile val ala ile asp pro arg asn ser asp thr
901  /   301                              931  /   311
ATT TAC AAC CCG CAA TTC AAA CAC AAG GCC AAA CTG ACT GCA GTC ACC TCC ACC AGC ACT
ile tyr asn pro gln phe lys his lys ala lys leu thr ala val thr ser thr ser thr
961  /   321                              991  /   331
GCC TAC ATG GAA CTC AAC AGC CTG ACA AAT GAG GAC TCT GCG GTC TAT TAC TGT ACC CCT
ala tyr met glu leu asn ser leu thr asn glu asp ser ala val tyr tyr cys thr pro
1021 /   341                              1051 /   351
CTT TAT TAC TTT GAC TCC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA CAT CAC CAT
leu tyr tyr phe asp ser trp gly gln gly thr thr leu thr val ser ser his his his
1081 /   361
TAG TAG
AMB AMB
```

met ser onc-Ang.

GbsFv

SEQ ID NOS:46,47

sFv onc metser -> 1-phase translation

DNA sequence 1065 b.p. GACATCAAGATG ... GTTGGATCTTGT linear

1 / 1 G-L sFv 31 / 11
GAC ATC AAG ATG ACC CAG TCT CCA TCT TCC ATG TAT GCA TCT CTA GGA GAG AGA GTC ACT
asp ile lys met thr gln ser pro ser ser met tyr ala ser leu gly glu arg val thr
61 / 21 91 / 31
TTC ACT TGC AAG GCG AGT CAG GAC ATT AAT AAC TAT TTA TGC TGG TTC CAG CAG AAA CCA
phe thr cys lys ala ser gln asp ile asn asn tyr leu cys trp phe gln gln lys pro
121 / 41 151 / 51
GGG AAA TCT CCT AAG ACC CTG ATC TAT CGT GCA AAC AGA CTG GTA GAT GGG GTC CCA TCA
gly lys ser pro lys thr leu ile tyr arg ala asn arg leu val asp gly val pro ser
181 / 61 211 / 71
AGG TTC AGT GGC AGT GGA TCT GGA CAA GAT TAT TCT CTC ACC ATT AGC AGC CTG GAG TAT GLsFv
arg phe ser gly ser gly ser gly gln asp tyr ser leu thr ile ser ser leu glu tyr
241 / 81 271 / 91
GAA GAT ATG GGA ATT TAT TAT TGT CTA CAG TAT GAT GAG TTT CCG TAC ACG TTC GGA GGG
glu asp met gly ile tyr tyr cys leu gln tyr asp glu phe pro tyr thr phe gly gly
301 / 101 331 / 111
GGG ACC AAG CTG GAA ATA AAA GGA GGC GGT GGC TCG GGC GGT GGC GGA TCG GGT GGC GGC
gly thr lys leu glu ile lys gly gly gly gly ser gly gly gly gly ser gly gly gly
361 / 121 391 / 131
GGC TCT GAG GTT CAG CTC CAG CAG TCT GGG ACT GTA CTG GCA AGG CCT GGG GCT TCA GTG
gly ser glu val gln leu gln gln ser gly thr val leu ala arg pro gly ala ser val
421 / 141 451 / 151
AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT TCC AGC TAC TGG ATG CAC TGG ATA AAA
lys met ser cys lys ala ser gly tyr thr phe ser ser tyr trp met his trp ile lys
481 / 161 511 / 171
CAG AGG CCT GGA CAG GGT CTG GAC TGG ATT GTC GCT ATT GAT CCT CGA AAT AGT GAT ACT
gln arg pro gly gln gly leu asp trp ile val ala ile asp pro arg asn ser asp thr
541 / 181 571 / 191
ATT TAC AAC CCG CAA TTC AAA CAC AAG GCC AAA CTG ACT GCA GTC ACC TCC ACC AGC ACT
ile tyr asn pro gln phe lys his lys ala lys leu thr ala val thr ser thr ser thr
601 / 201 631 / 211
GCC TAC ATG GAA CTC AAC AGC CTG ACA AAT GAG GAC TCT GCG GTC TAT TAC TGT ACC CCT
ala tyr met glu leu asn ser leu thr asn glu asp ser ala val tyr tyr cys thr pro
661 / 221 691 / 231 FB
CTT TAT TAC TTT GAC TCC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAG AAA
leu tyr tyr phe asp ser trp gly gln gly thr thr leu thr val ser ser ala lys lys
721 / 241 ~~751 / 251~~ met dia onc
CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA
leu asn asp ala gln ala pro lys ser asp met ser asp trp leu thr phe gln lys lys
781 / 261 811 / 271
CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC
his ile thr asn thr arg asp val asp cys asp asn ile met ser thr asn leu phe his
841 / 281 871 / 291
TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA met dia onc
cys lys asp lys asn thr phe ile tyr ser arg pro glu pro val lys ala ile cys lys
901 / 301 931 / 311
GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT
gly ile ile ala ser lys asn val leu thr thr ser glu phe tyr leu ser asp cys asn
961 / 321 991 / 331
GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT
val thr ser arg pro cys lys tyr lys leu lys lys ser thr asn lys phe cys val thr
1021 / 341 1051 / 351
TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT
cys glu asn gln ala pro val his phe val gly val gly ser cys

SEQ ID NOS:48,49

met glu Cnc-FB E6
~~Nerf~~-FB-E6 -> 1-phase Translation

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DNA  sequence    1074 b.p.   ATGGAGGATTGG ... TCACATCACCAT   linear
     met gln cnc
1    /  1                                 31   /  11
ATG GAG GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT
met glu asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp cys
61   ser 21                               91   /  31                              met gln cnc
GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA
asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr ser
121  /  41                               151  /  51
CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT
arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu thr
181  /  61                               211  /  71
ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA
thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys leu
241  /  81                               271  /  91
AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT
lys lys ser thr asn lys phe cys val thr cys glu asn gln ala pro val his phe val
301  /  101               FB             331  /  111                   E6 sFv
GGA GTT GGA TCT TGT GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT GAC ATC
gly val gly ser cys ala lys lys leu asn asp ala gln ala pro lys ser asp asp ile
361  /  121                              391  /  131
AAG ATG ACC CAG TCT CCA TCT TCC ATG TAT GCA TCT CTA GGA GAG AGA GTC ACT TTC ACT
lys met thr gln ser pro ser ser met tyr ala ser leu gly glu arg val thr phe thr
421  /  141                              451  /  151
TGC AAG GCG AGT CAG GAC ATT AAT AAC TAT TTA TGC TGG TTC CAG CAG AAA CCA GGG AAA
cys lys ala ser gln asp ile asn asn tyr leu cys trp phe gln gln lys pro gly lys
481  /  161                              511  /  171
TCT CCT AAG ACC CTG ATC TAT CGT GCA AAC AGA CTG GTA GAT GGG GTC CCA TCA AGG TTC
ser pro lys thr leu ile tyr arg ala asn arg leu val asp gly val pro ser arg phe
541  /  181                              571  /  191
AGT GGC AGT GGA TCT GGA CAA GAT TAT TCT CTC ACC ATT AGC AGC CTG GAG TAT GAA GAT
ser gly ser gly ser gly gln asp tyr ser leu thr ile ser ser leu glu tyr glu asp
601  /  201                              631  /  211                              C6 sFv
ATG GGA ATT TAT TAT TGT CTA CAG TAT GAT GAG TTT CCG TAC ACG TTC GGA GGG GGG ACC
met gly ile tyr tyr cys leu gln tyr asp glu phe pro tyr thr phe gly gly gly thr
661  /  221                              691  /  231
AAG CTG GAA ATA AAA GGA GGC GGT GGC TCG GGC GGT GGC GGA TCG GGT GGC GGC GGC TCT
lys leu glu ile lys gly gly gly gly ser gly gly gly gly ser gly gly gly gly ser
721  /  241                              751  /  251
GAG GTT CAG CTC CAG CAG TCT GGG ACT GTA CTG GCA AGG CCT GGG GCT TCA GTG AAG ATG
glu val gln leu gln gln ser gly thr val leu ala arg pro gly ala ser val lys met
781  /  261                              811  /  271
TCC TGC AAG GCT TCT GGC TAC ACC TTT TCC AGC TAC TGG ATG CAC TGG ATA AAA CAG AGG
ser cys lys ala ser gly tyr thr phe ser ser tyr trp met his trp ile lys gln arg
841  /  281                              871  /  291
CCT GGA CAG GGT CTG GAC TGG ATT GTC GCT ATT GAT CCT CGA AAT AGT GAT ACT ATT TAC
pro gly gln gly leu asp trp ile val ala ile asp pro arg asn ser asp thr ile tyr
901  /  301                              931  /  311
AAC CCG CAA TTC AAA CAC AAG GCC AAA CTG ACT GCA GTC ACC TCC ACC AGC ACT GCC TAC
asn pro gln phe lys his lys ala lys leu thr ala val thr ser thr ser thr ala tyr
961  /  321                              991  /  331
ATG GAA CTC AAC AGC CTG ACA AAT GAG GAC TCT GCG GTC TAT TAC TGT ACC CCT CTT TAT
met glu leu asn ser leu thr asn glu asp ser ala val tyr tyr cys thr pro leu tyr
1021 /  341                              1051 /  351
TAC TTT GAC TCC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA CAT CAC CAT
tyr phe asp ser trp gly gln gly thr thr leu thr val ser ser his his his
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SEQ ID NOS:50,51

MacsequenceA57FBMOC31 -> 1-phase Translation 73

DNA sequence 1095 b.p. ATGTCAGATTGG ... GCGGCCGCCGCA linear

met alu onc A87

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1    /   1                                         31   /   11
ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT
met ser asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp cys
61   /   21                                        91   /   31
GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA
asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr ser
121  /   41                                        151  /   51
CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT
arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu thr
181  /   61                                        211  /   71
ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA
thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys leu
241  /   81                                        271  /   91
AAG AAA TCA ACT AAT AAA TTT GCT GTA ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT
lys lys ser thr asn lys phe ala val thr cys glu asn gln ala pro val his phe val
301  /   101                   FS                  331  /   111                 MOC31
GGA GTT GGA TCT TGT GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT CAG GTG
gly val gly ser cys ala lys lys leu asn asp ala gln ala pro lys ser asp gln val
361  /   121                                       391  /   131
AAG CTG CAG CAG TCA GGA CCT GAG CTG AAG AAG CCT GGA GAG ACA GTC AAG ATC TCC TGC
lys leu gln gln ser gly pro glu leu lys lys pro gly glu thr val lys ile ser cys
421  /   141                                       451  /   151
AAG GCT TCT GGG TAC ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA
lys ala ser gly tyr thr phe thr asn tyr gly met asn trp val lys gln ala pro gly
481  /   161                                       511  /   171
AAG GGT TTA AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG TCA ACA TAT GCT GAT
lys gly leu lys trp met gly trp ile asn thr tyr thr gly glu ser thr tyr ala asp
541  /   181                                       571  /   191
GAC TTC AAG GGA CGG TTT GCC TTT TCT CTA GAA ACC TCT GCC AGC GCT GCC TAT TTG CAG
asp phe lys gly arg phe ala phe ser leu glu thr ser ala ser ala ala tyr leu gln
601  /   201                                       631  /   211
ATC AAC AAC CTC AAA AAT GAG GAC ACG GCT ACA TAT TTC TGT GCA AGA TTC GCT ATT AAG
ile asn asn leu lys asn glu asp thr ala thr tyr phe cys ala arg phe ala ile lys
661  /   221                                       691  /   231
GGG GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC
gly asp tyr trp gly gln gly thr thr val thr val ser ser gly gly gly gly ser gly
721  /   241                                       751  /   251
GGA GGT GGC TCT GGC GGT GGC GGA TCG GAC ATT GTG CTA ACC CAG TCT CCA TTC TCC AAT
gly gly gly ser gly gly gly gly ser asp ile val leu thr gln ser pro phe ser asn
781  /   261                                       811  /   271
CCA GTC ACT CTT GGA ACA TCA GCT TCC ATC TCC TGC AGG TCT ACT AAG AGT CTC CTA CAT
pro val thr leu gly thr ser ala ser ile ser cys arg ser thr lys ser leu leu his
841  /   281                                       871  /   291
AGT AAT GGC ATC ACT TAT TTG TAT TGG TAT CTG CAG AAG CCA GGC CAG TCT CCT CAG CTC
ser asn gly ile thr tyr leu tyr trp tyr leu gln lys pro gly gln ser pro gln leu
901  /   301                                       931  /   311
CTG ATT TAT CAG ATG TCC AAC CTT GCC TCA GGA GTC CCA GAC AGG TTC AGT AGC AGT GGG
leu ile tyr gln met ser asn leu ala ser gly val pro asp arg phe ser ser ser gly
961  /   321                                       991  /   331
TCA GGA ACT GAT TTC ACA CTG AGA ATC AGC AGA GTG GAG GCT GAG GAT GTG GGT GTT TAT
ser gly thr asp phe thr leu arg ile ser arg val glu ala glu asp val gly val tyr
1021 /   341                                       1051 /   351
TAC TGT GCT CAA AAT CTA GAA ATT CCT CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC
tyr cys ala gln asn leu glu ile pro arg thr phe gly gly gly thr lys leu glu ile
1081 /   361
AAA CGG GCG GCC GCA
lys arg ala ala ala
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met alu onc A87

MOC31 sFv

SEQ ID NOS:52,53

MOC31FBmetserOnc -> 1-phase Translation 74

DNA sequ nce 1098 b.p. GGTCAGGTGAAG ... GTTGGATCTTGT linear

MOC31sFv

1 / 1 31 / 11
GGT CAG GTG AAG CTG CAG CAG TCA GGA CCT GAG CTG AAG AAG CCT GGA GAG ACA GTC AAG
gly gln val lys leu gln gln ser gly pro glu leu lys lys pro gly glu thr val lys
61 / 21 91 / 31
ATC TCC TGC AAG GCT TCT GGG TAC ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG AAG CAG
ile ser cys lys ala ser gly tyr thr phe thr asn tyr gly met asn trp val lys gln
121 / 41 151 / 51
GCT CCA GGA AAG GGT TTA AAG TGG ATG GGC TGG ATA AAC ACC TAC ACT GGA GAG TCA ACA
ala pro gly lys gly leu lys trp met gly trp ile asn thr tyr thr gly glu ser thr
181 / 61 211 / 71
TAT GCT GAT GAC TTC AAG GGA CGG TTT GCC TTT TCT CTA GAA ACC TCT GCC AGC GCT GCC
tyr ala asp asp phe lys gly arg phe ala phe ser leu glu thr ser ala ser ala ala
241 / 81 271 / 91
TAT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC ACG GCT ACA TAT TTC TGT GCA AGA TTC
tyr leu gln ile asn asn leu lys asn glu asp thr ala thr tyr phe cys ala arg phe MOC31sFv
301 / 101 331 / 111
GCT ATT AAG GGG GAC TAC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC
ala ile lys gly asp tyr trp gly gln gly thr thr val thr val ser ser gly gly gly
361 / 121 391 / 131
GGT TCA GGC GGA GGT GGC TCT GGC GGT GGC GGA TCG GAC ATT GTG CTA ACC CAG TCT CCA
gly ser gly gly gly gly ser gly gly gly gly ser asp ile val leu thr gln ser pro
421 / 141 451 / 151
TTC TCC AAT CCA GTC ACT CTT GGA ACA TCA GCT TCC ATC TCC TGC AGG TCT ACT AAG AGT
phe ser asn pro val thr leu gly thr ser ala ser ile ser cys arg ser thr lys ser
481 / 161 511 / 171
CTC CTA CAT AGT AAT GGC ATC ACT TAT TTG TAT TGG TAT CTG CAG AAG CCA GGC CAG TCT
leu leu his ser asn gly ile thr tyr leu tyr trp tyr leu gln lys pro gly gln ser
541 / 181 571 / 191
CCT CAG CTC CTG ATT TAT CAG ATG TCC AAC CTT GCC TCA GGA GTC CCA GAC AGG TTC AGT
pro gln leu leu ile tyr gln met ser asn leu ala ser gly val pro asp arg phe ser
601 / 201 631 / 211
AGC AGT GGG TCA GGA ACT GAT TTC ACA CTG AGA ATC AGC AGA GTG GAG GCT GAG GAT GTG
ser ser gly ser gly thr asp phe thr leu arg ile ser arg val glu ala glu asp val
661 / 221 691 / 231
GGT GTT TAT TAC TGT GCT CAA AAT CTA GAA ATT CCT CGG ACG TTC GGT GGA GGC ACC AAG
gly val tyr tyr cys ala gln asn leu glu ile pro arg thr phe gly gly gly thr lys
721 / 241 FB 751 / 251
CTG GAA ATC AAA CGG GCG GCC GCA GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT
leu glu ile lys arg ala ala ala ala lys lys leu asn asp ala gln ala pro lys ser
781 / 261 metseronc 811 / 271
GAT ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT
asp met ser asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp
841 / 281 871 / 291
TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT
cys asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr
901 / 301 931 / 311
TCA CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA
ser arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu metseronc
961 / 321 991 / 331
ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA
thr thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys
1021 / 341 1051 / 351
TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT
leu lys lys ser thr asn lys phe cys val thr cys glu asn gln ala pro val his phe
1081 / 361
GTT GGA GTT GGA TCT TGT
val gly val gly ser cys

SEQ ID NOS:54,55

MetserOncFBMOC161 -> 1-phase Translati n 75

DNA sequ nce 1065 b.p. ATGTCAGATTGG ... GAGCTGAAACGG linear

met Rec onc

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1   /   1                                   31  /  11
ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT
met ser asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp cys
61  /  21                                   91  /  31
GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA
asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr ser
121 /  41                                   151 /  51
CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT
arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu thr
181 /  61                                   211 /  71
ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA
thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys leu
241 /  81                                   271 /  91
AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT
lys lys ser thr asn lys phe cys val thr cys glu asn gln ala pro val his phe val
301 / 101           FB                    331 / 111
GGA GTT GGA TCT TGT GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT CAG GTC
gly val gly ser cys ala lys lys leu asn asp ala gln ala pro lys ser asp gln val
361 / 121                                   391 / 131
CAA CTG CAG CAG TCA GGA ACT GAG CTG ATA AGG CCT GGG ACT TCA GTG AAG ATA TCC TGT
gln leu gln gln ser gly thr glu leu ile arg pro gly thr ser val lys ile ser cys
421 / 141                                   451 / 151
AAG GCT TCT GGA TAC GCC TTC ACT GAC TAC TGG CTA GGT TGG GTA AAA CAC AGG CCT GGA
lys ala ser gly tyr ala phe thr asp tyr trp leu gly trp val lys his arg pro gly
481 / 161                                   511 / 171
CAT GGA CTT GAG TGG ATT GGA GAT ATT TAC CCT GGA AGT GAT AAT ACT TAC TAC AAT GAG
his gly leu glu trp ile gly asp ile tyr pro gly ser asp asn thr tyr tyr asn glu
541 / 181                                   571 / 191
AAA TTC AAG GGC AAA GCC ACA CTG ACT ACA GAC AAA TCC TCG AGC ACA GCC TAT ATG CAG
lys phe lys gly lys ala thr leu thr thr asp lys ser ser ser thr ala tyr met gln
601 / 201                                   631 / 211
CTC AGT AGC CTG ACA TCT GAG GAC TCT GCT GTC TAT TTC TGT GCA AGG GGC CTT AAA GGA
leu ser ser leu thr ser glu asp ser ala val tyr phe cys ala arg gly leu lys gly
661 / 221                                   691 / 231
GAC TAC TGG GGC CAA GGG ACC ACC GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC GGA
asp tyr trp gly gln gly thr thr val thr val ser ser gly gly gly gly ser gly gly
721 / 241                                   751 / 251
GGT GGC TCT GGC GGT GGC GGA TCG GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT
gly gly ser gly gly gly gly ser asp ile gln met thr gln ser pro ser ser leu ser
781 / 261                                   811 / 271
GCA TCT CTG GGA GGC AAA GTC ACC ATC ACT TGC AAG GCA AGC CAA GAC ATT AAG AAG TCT
ala ser leu gly gly lys val thr ile thr cys lys ala ser gln asp ile lys lys ser
841 / 281                                   871 / 291
ATA GCT TGG TAC CAA CAC AAG CCT GGA AAA GGT CCT AGG CTG CTC ATT CAT TAC ACA TCT
ile ala trp tyr gln his lys pro gly lys gly pro arg leu leu ile his tyr thr ser
901 / 301                                   931 / 311
ACA TTA CAG CCA GGC ATC CCA TCA AGG TTC AGT GGA AGT GGG TCT GGT GAA GAA TAT TCC
thr leu gln pro gly ile pro ser arg phe ser gly ser gly ser gly glu glu tyr ser
961 / 321                                   991 / 331
TTC AGC ATC AGC AAC CTG GAG CCT GAA GAT ATT GCA ACT TAT TAT TGT CAA CAG TAT GAT
phe ser ile ser asn leu glu pro glu asp ile ala thr tyr tyr cys gln gln tyr asp
1021 / 341                                  1051 / 351
AAT CTT CGG ACG TTC GGT GGA GGC ACC AAG CTG GAG CTG AAA CGG
asn leu arg thr phe gly gly gly thr lys leu glu leu lys arg
```

met Rec onc

Hoc161SFv

Hoc161SFv

SEQ ID NOS:56,57

IL2FBMetserOnc -> 1-phase Translation


```
DNA  sequ nce   753 b.p.   GCACCTACTTCA ... GTTGGATCTTGT   linear
        IL2
1   /   1                                 31   /   11
GCA CCT ACT TCA ACT TCT ACA AAG AAA ACA CAG CTA CAA CTG GAG CAT TTA CTG CTG GAT
ala pro thr ser thr ser thr lys lys thr gln leu gln leu glu his leu l u leu asp
61   /   21                               91   /   31
TTA CAG ATG ATT TTG AAT GGA ATT AAT AAT TAC AAG AAT CCC AAA CTC ACC AGG ATG CTC
leu gln met ile leu asn gly ile asn asn tyr lys asn pro lys leu thr arg met leu      IL2
121  /   41                               151  /   51
ACA TTT AAG TTT TAC ATG CCC AAG AAG GCC ACA GAA CTG AAA CAT CTT CAG TGT CTA GAA
thr phe lys phe tyr met pro lys lys ala thr glu leu lys his leu gln cys leu glu
181  /   61                               211  /   71
GAA GAA CTC AAA CCT CTG GAG GAA GTG CTA AAT TTA GCT CAA AGC AAA AAC TTT CAC TTA
glu glu leu lys pro leu glu glu val leu asn leu ala gln ser lys asn phe his leu
241  /   81                               271  /   91
AGA CCC AGG GAC TTA ATC AGC AAT ATC AAC GTA ATA GTT CTG GAA CTA AAG GGA TCT GAA
arg pro arg asp leu ile ser asn ile asn val ile val leu glu leu lys gly ser glu
301  /  101                               331  /  111
ACA ACA TTC ATG TGT GAA TAT GCT GAT GAG ACA GCA ACC ATT GTA GAA TTT CTG AAC AGA
thr thr phe met cys glu tyr ala asp glu thr ala thr ile val glu phe leu asn arg
361  /  121                               391  / 131         FB
TGG ATT ACC TTT TGT CAA AGC ATC ATC TCA ACA CTG ACT GCC AAG AAA CTG AAC GAC GCT
trp ile thr phe cys gln ser ile ile ser thr leu thr ala lys lys leu asn asp ala
421  /  141                        met ser onc          met ser onc
CAG GCG CCG AAG AGT GAT ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC
gln ala pro lys ser asp met ser asp trp leu thr phe gln lys lys his ile thr asn
481  /  161                               511  /  171
ACA AGG GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG      met ser onc
thr arg asp val asp cys asp asn ile met ser thr asn leu phe his cys lys asp lys
541  /  181                               571  /  191
AAC ACT TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC
asn thr phe ile tyr ser arg pro glu pro val lys ala ile cys lys gly ile ile ala
601  /  201                               631  /  211
TCC AAA AAT GTC TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG
ser lys asn val leu thr thr ser glu phe tyr leu ser asp cys asn val thr ser arg
661  /  221                               691  /  231
CCT TGC AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG
pro cys lys tyr lys leu lys lys ser thr asn lys phe cys val thr cys glu asn gln
721  /  241                               751  /  251
GCA CCA GTT CAT TTT GTT GGA GTT GGA TCT TGT
ala pro val his phe val gly val gly ser cys
```

SEQ ID NOS:58,59

MetserOncFBIL2 -> 1-phase Translation

DNA sequence 768 b.p. ATGTCAGATTGG ... CACCATTAATAG linear

```
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ATG TCA GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG GAT GTT GAT TGT
met ser asp trp leu thr phe gln lys lys his ile thr asn thr arg asp val asp cys
61  /   21                                 91   /   31
GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT TTT ATC TAT TCA
asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr phe ile tyr ser
121 /   41                                 151  /   51
CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA AAT GTG TTA ACT
arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys asn val leu thr
181 /   61                                 211  /   71
ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC AAG TAT AAA TTA
thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys lys tyr lys leu
241 /   81                                 271  /   91
AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA GTT CAT TTT GTT
lys lys ser thr asn lys phe cys val thr cys glu asn gln ala pro val his phe val
301 /   101                                331  /   111
GGA GTT GGA TCT TGT GCC AAG AAA CTG AAC GAC GCT CAG GCG CCG AAG AGT GAT GCA CCT
gly val gly ser cys ala lys lys leu asn asp ala gln ala pro lys ser asp ala pro
361 /   121                                391  /   131
ACT TCA ACT TCT ACA AAG AAA ACA CAG CTA CAA CTG GAG CAT TTA CTG CTG GAT TTA CAG
thr ser thr ser thr lys lys thr gln leu gln leu glu his leu leu leu asp leu gln
421 /   141                                451  /   151
ATG ATT TTG AAT GGA ATT AAT AAT TAC AAG AAT CCC AAA CTC ACC AGG ATG CTC ACA TTT
met ile leu asn gly ile asn asn tyr lys asn pro lys leu thr arg met leu thr phe
481 /   161                                511  /   171
AAG TTT TAC ATG CCC AAG AAG GCC ACA GAA CTG AAA CAT CTT CAG TGT CTA GAA GAA GAA
lys phe tyr met pro lys lys ala thr glu leu lys his leu gln cys leu glu glu glu
541 /   181                                571  /   191
CTC AAA CCT CTG GAG GAA GTG CTA AAT TTA GCT CAA AGC AAA AAC TTT CAC TTA AGA CCC
leu lys pro leu glu glu val leu asn leu ala gln ser lys asn phe his leu arg pro
601 /   201                                631  /   211
AGG GAC TTA ATC AGC AAT ATC AAC GTA ATA GTT CTG GAA CTA AAG GGA TCT GAA ACA ACA
arg asp leu ile ser asn ile asn val ile val leu glu leu lys gly ser glu thr thr
661 /   221                                691  /   231
TTC ATG TGT GAA TAT GCT GAT GAG ACA GCA ACC ATT GTA GAA TTT CTG AAC AGA TGG ATT
phe met cys glu tyr ala asp glu thr ala thr ile val glu phe leu asn arg trp ile
721 /   241                                751  /   251
ACC TTT TGT CAA AGC ATC ATC TCA ACA CTG ACT CAT CAC CAT TAA TAG
thr phe cys gln ser ile ile ser thr leu thr his his his OCH AMB
```

met ser onc

FB

IL2

IL2

SEQ ID NOS:60,61

SIG PEP ONC -> 1-phase Translation

DNA sequence 387 b.p. ATGGGTCTGGAA ... GTTGGATCTTGT linear

```
1 / 1 Signal peptide                     31 / 11
ATG GGT CTG GAA AAA TCT CTG ATC CTG TTC CCG CTG TTC TTC CTG CTG CTG GGT TGG GTT
met gly leu glu lys ser leu ile leu phe pro leu phe phe leu leu leu gly trp val
61 / 21                                91 / 31
CAG CCG TCT CTG GGT CAG GAT TGG CTT ACA TTT CAG AAA AAA CAC ATC ACA AAC ACA AGG
gln pro ser leu gly gln asp trp leu thr phe gln lys lys his ile thr asn thr arg
121 / 41                               151 / 51
GAT GTT GAT TGT GAT AAT ATC ATG TCA ACA AAC TTG TTC CAC TGC AAG GAC AAG AAC ACT
asp val asp cys asp asn ile met ser thr asn leu phe his cys lys asp lys asn thr
181 / 61                               211 / 71
TTT ATC TAT TCA CGT CCT GAG CCA GTG AAG GCC ATC TGT AAA GGA ATT ATA GCC TCC AAA
phe ile tyr ser arg pro glu pro val lys ala ile cys lys gly ile ile ala ser lys
241 / 81                               271 / 91
AAT GTG TTA ACT ACC TCT GAG TTT TAT CTC TCT GAT TGC AAT GTA ACA AGC AGG CCT TGC
asn val leu thr thr ser glu phe tyr leu ser asp cys asn val thr ser arg pro cys
301 / 101                              331 / 111
AAG TAT AAA TTA AAG AAA TCA ACT AAT AAA TTT TGT GTA ACT TGT GAA AAT CAG GCA CCA
lys tyr lys leu lys lys ser thr asn lys phe cys val thr cys glu asn gln ala pro
361 / 121
GTT CAT TTT GTT GGA GTT GGA TCT TGT
val his phe val gly val gly ser cys
```

SEQ ID NOS:62,63

WHAT IS CLAIMED IS:

1. A ribonuclease molecule that has (a) measurable ribonuclease activity; (b) an amino terminal end beginning with a methionine which is followed by any amino acid other than glutamic acid; (c) a cysteine at amino acid positions 26, 40, 58, 84, 95 and 110; a lysine at position 41 and a histidine at position 119, such positions being determined with reference to those specified amino acid positions of bovine RNase A (SEQ ID NO:13), and (d) an nOnc-derived amino acid sequence.

2. The ribonuclease of claim 1 which has an amino terminal end selected from the group consisting of: Met-Lys; Met-Tyr; Met-Ser; Met-Ala; Met-Arg; and Met-Asn.

3. The ribonuclease of claim 1, which has an amino terminal end selected from the group consisting of:

Met-Ala;
Met-Ala-Ala;
Met-Ala-Ala-Ser;
Met-Arg;
Met-(J);
Met-Lys-(J);
Met-Arg-(J);
Met-Lys;
Met-Lys-Pro;
Met-Lys-(J)-Pro (SEQ ID NO:14);
Met-Lys-Pro-(J) (SEQ ID NO:15);
Met-Asn;
Met-Gln;
Met-Asn-(J);
Met-Gln-(J);
Met-Asn-(J)-Pro (SEQ ID NO:16);
Met-(J)-Lys;
Met-(J)-Lys-Pro (SEQ ID NO:17); and
Met-(J)-Pro-Lys (SEQ ID NO:18);

where (J) is Ser, Tyr or Thr.

4. The ribonuclease of claim 1, which has an amino terminal end of Met-Ala.

5. The ribonuclease of claim 1, which has an amino terminal end of Met-Arg.

6. The ribonuclease of claim 1, which has an amino terminal end of Met-Lys.

7. The ribonuclease of claim 1, which has an amino terminal end of Met-Asn.

8. The ribonuclease of claim 1, which has an amino terminal end of Met-Gln.

9. The ribonuclease of claim 1, which has an amino terminal end selected from the group consisting of Met-Ser; Met-Tyr or Met-Thr.

10. The ribonuclease of claim 3, wherein aspartic acid of amino acid position 2 of nOnc (position 4 with reference to the sequence of bovine RNase) is deleted or replaced by Ala or Asn.

11. The ribonuclease of claim 1, comprising a molecule having an amino terminal end encoded by a sequence derived from the amino terminal end of EDN which is followed by a sequence from rOnc.

12. The ribonuclease of claim 11 wherein the amino acid sequence is one selected from the group consisting of those sequences substantially identical to those of a formula:

$$Met(-1)EDN_{(1-m)}Onc_{(n-104)}$$

wherein Met(-1) refers to an amino terminal residue of Met; wherein $EDN_{(1-m)}$ refers to a contiguous sequence of amino acids of a length beginning at amino acid position 1 of EDN (SEQ ID NO:9) and continuing to and including amino acid position "m" of EDN; wherein $Onc_{(n-104)}$ refers to a sequence of contiguous amino acids beginning at amino acid position "n" and continuing to and including amino acid position 104 as set out in SEQ ID NO:1; such that:

when m is 21, n is 16 or 17;
when m is 22, n is 17;
when m is 20, n is 16;
when m is 19, n is 15;
when m is 18, n is 14;
when m is 17, n is 12 or 13;
when m is 16, n is 11, 12, 13 or 14;
when m is 15, n is 10;
when m is 14, n is 9;
when m is 13, n is 8; and
when m is 5, n is 1.

13. The ribonuclease of claim 1, comprising an amino acid sequence substantially identical to that of SEQ ID NO:28.

14. The ribonuclease of claim 1, comprising an amino acid sequence substantially identical to that of SEQ ID NO:22.

15. The ribonuclease of claim 1, comprising an amino acid sequence substantially identical to that of SEQ ID NO:24.

16. The ribonuclease of claim 1, comprising an amino acid sequence substantially identical to that of SEQ ID NO:26.

17. The ribonuclease of claim 1, comprising an amino acid sequence substantially identical to that of SEQ ID NO:30.

18. The ribonuclease of claim 1, comprising an amino acid sequence substantially identical to that of SEQ ID NO:32.

19. The ribonuclease of claim 1, which includes an amino acid sequence substantially identical to that of SEQ ID NO:2.

20. The ribonuclease of claim 1, comprising a carboxyl terminal end derived from angiogenin.

21. The ribonuclease of claim 20, comprising an amino acid sequence substantially identical to that of SEQ ID NO:20.

22. An amino acid sequence substantially identical to that set out in SEQ ID NO:2.

23. The ribonuclease of claim 1 joined to a ligand binding moiety or a label.

24. The ribonuclease molecule of claim 23, wherein the molecule is joined to an antibody.

25. A nucleic acid sequence encoding the amino acid sequence of claim 1.

26. A pharmaceutical composition comprising a cytotoxic amount of a ribonuclease of claim 1 and a pharmaceutically acceptable carrier.

27. The pharmaceutical composition of claim 26 wherein the ribonuclease is joined to a ligand binding moiety.

28. A method of selectively killing cells comprising contacting cells to be killed with a ribonuclease of claim 1 joined to a ligand binding moiety.

29. The ribonuclease molecule of claim 1 which further has a nuclear localization signal.

30. The ribonuclease molecule of claim 1 which further has an endoplasmic retention sequence.

31. A vector comprising a nucleic acid encoding a ribonuclease of claim 1.

32. A host cell comprising a nucleic acid encoding a ribonuclease of claim 1.

Onconase	EDWLTFQKKH	ITNTRDVDCD	NIMSTNLFHC	KDKNTFIYSR	PEPVKAICKG	50
Rana clone 9	....	......DVDCD	NIMSTNLFHC	KDKNTFIYSR	PEPVKAICKG	35
Onconase	IIASKNVLTT	SEFYLSDCNV	TSRPCKYKLK	KSTNKFCVTC	ENQAPVHFVG	100
Rana clone 9	IIASKNVLTT	SEFYLSDCNV	TSRPCKYKLK	KSTNKFCVTC	ENQAPVHF..	83
Onconase	VGSC					104
Rana clone 9	....					83

Fig. 1

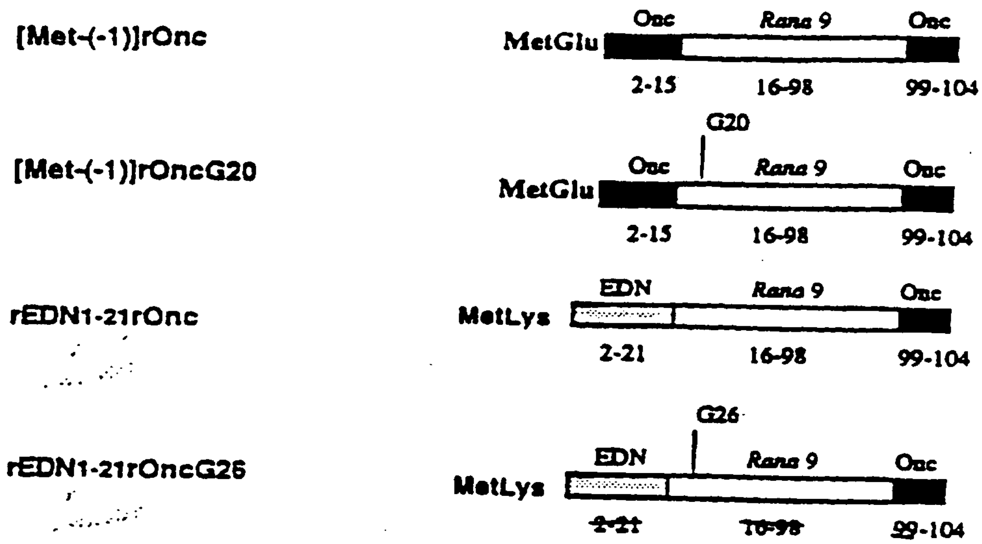


Fig. 2A

Onc	.....edwlt	fqkkhi.tnt	rDVD.....CD	NIMSTNLF..
EDN	kppqftwaqw	fetqhinmts	qq.......ct	namqvinnyq
OncG20	.....edwlt	fqkkhi.tnt	rDVD.....CC	NIMSTNLF..
EDN1-21Onc	kppqftwaqw	fetqhinmts	qDVD.....CD	NIMSTNLF..
EDN1-21OncG26	kppqftwaqw	fetqhinmts	qDVD.....CC	NIMSTNLF..

Fig. 2B

Fig. 3A

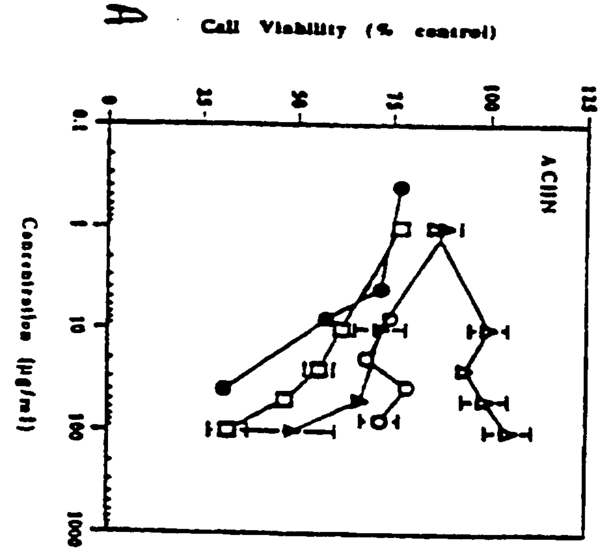


Fig. 3B

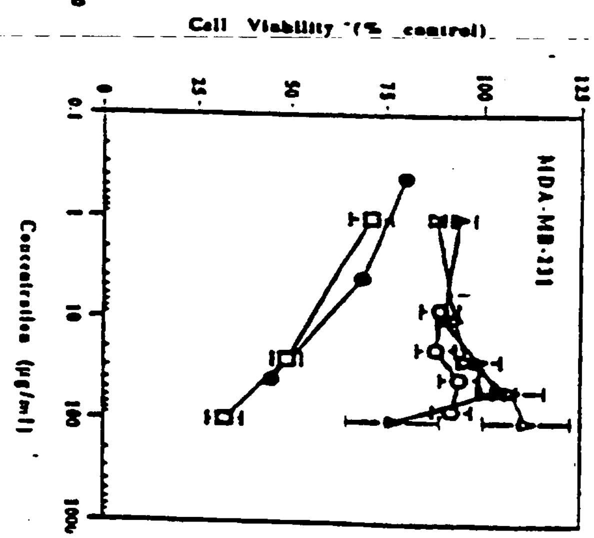


Fig. 3C

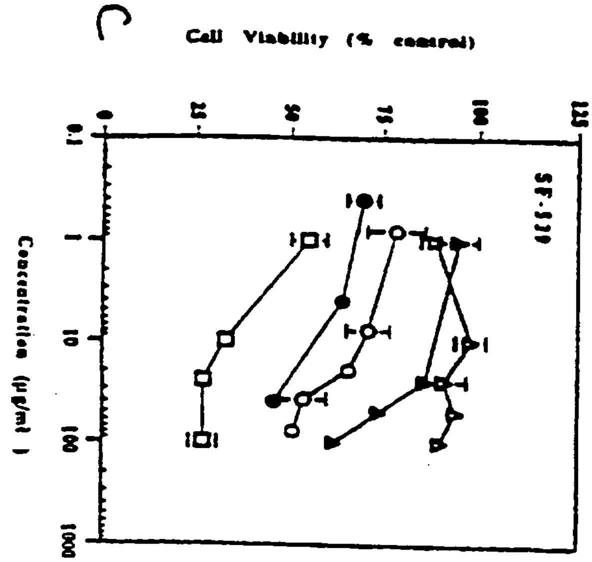
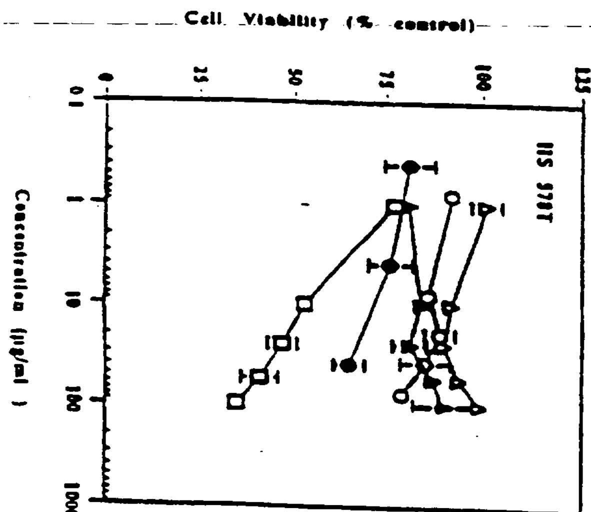


Fig. 3D



Figs 3

	1	•			• 50
Frog Lectin	.....enwat	FqqkHi.int	piin....Cn	tiMdnniyiv	ggqCKrvNTF
Onconase	.....edwlt	FqkkHi.tnt	rdvd....Cd	niMstnlf..	..hCKdkNTF
EDN	kppqftwaqw	FetqHinmts	qq......Ct	naMqvinnyq	rr.CKnqNTF
ECP	rppqftraqw	FaiqHislnp	pr......Ct	iaMrainnyr	wr.CKnqNTF
Ang	.aqddyryih	FltqHyd.ak	pkgrndeyCf	hmMknrrltr	p..CKdrNTF
Seminal	..kes.aaak	FerqHmdsgn	spsssnyCn	lmMccrkmtq	gk.CKpvNTF
Rnase A	..ket.aaak	FerqHmdsst	saasssnyCn	qmMksrnltk	dr.CKpvNTF
	51				100
Frog Lectin	iissattvka	iCtgvi..nm	nvl.......	SttrfqlntC	trts...itp
Onconase	iysrpepvka	iCkgii.ask	nvlt.....t	Sefy..lsdC	......nvts
EDN	llttfanvvn	vCgnpnmtcp	snktrknchh	SgsqvplihC	nlttpspqni
ECP	lrttfanvvn	vCgnqsircp	hnrtlnnchr	SrfrvpllhC	dlinpgaqni
Ang	ihgnkndika	iCedrngqpy	rg....dlri	SksefqitiC	khkggs..sr
Seminal	vhesladvka	vCsqkkvtck	ngqt..ncyq	SkstmritdC	ret..gssky
Rnase A	vhesladvqa	vCsqknvack	ngqt..ncyq	SystmsitdC	ret..gssky
	101			•	150
Frog Lectin	rpCpYssrte	tnyicVkCen	q.........	..yPVHfagi	grcp......
Onconase	rpCkYklkks	tnkfcVtCen	q.........	..aPVHfvgv	gsc.......
EDN	snCrYaqtpa	nmfyiVaCdn	rdqrrdppqy	pvvPVHldri	i.........
ECP	snCrYadrpg	rrfyvVaCdn	rd.prdspry	pvvPVHldtt	i.........
Ang	ppCrYgated	srvivVgCen	g.........	..lPVHfdes	fitprn....
Seminal	pnCaYkttqv	ekhiiVaCgg	k.........	psvPVHfdas	v.........
Rnase A	pnCaYkttqa	nkhiiVaCeg	n.........	pyvPVHfdas	v.........

Fig. 4

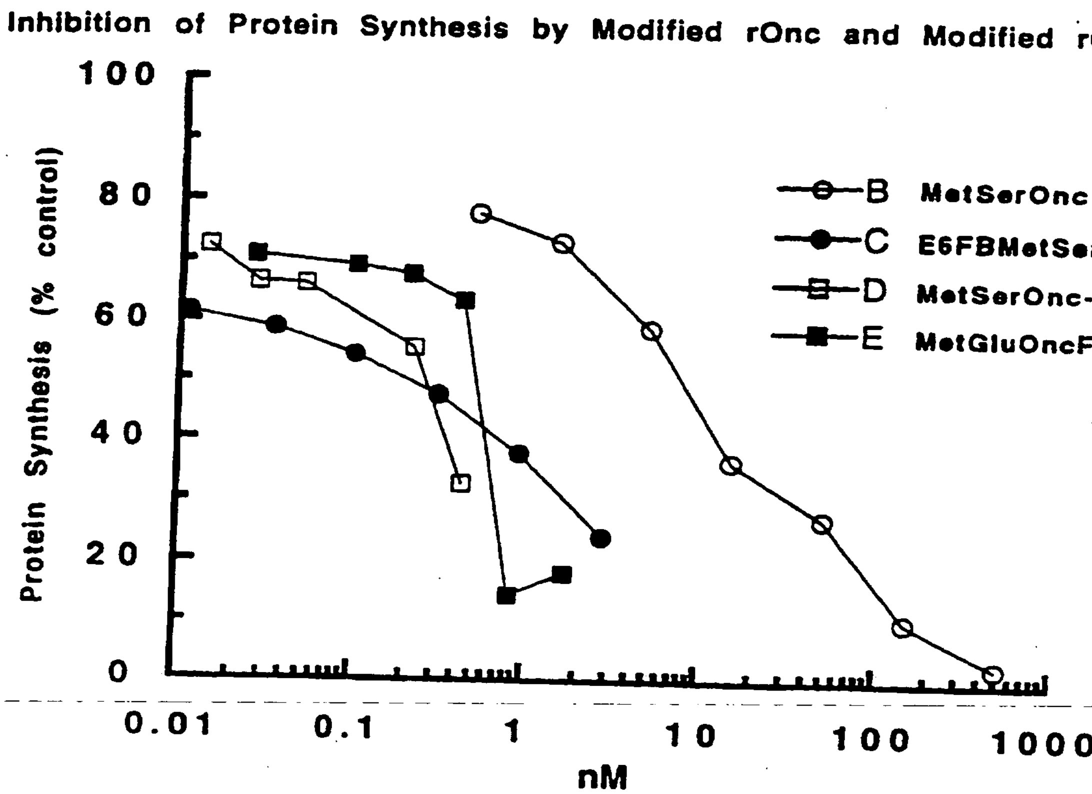

Inhibition of Protein Synthesis by Modified rOnc and Modified rOnc-sFvs
Protein Synthesis (% control)
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
nM
B MetSerOnc
C E6FBMetSerOnc
D MetSerOnc-AngFBE6
E MetGluOncFBE6

Figure 5

Inhibiti f cell viability by M tLysTyrOn d M tAlaAlaTyrOnc

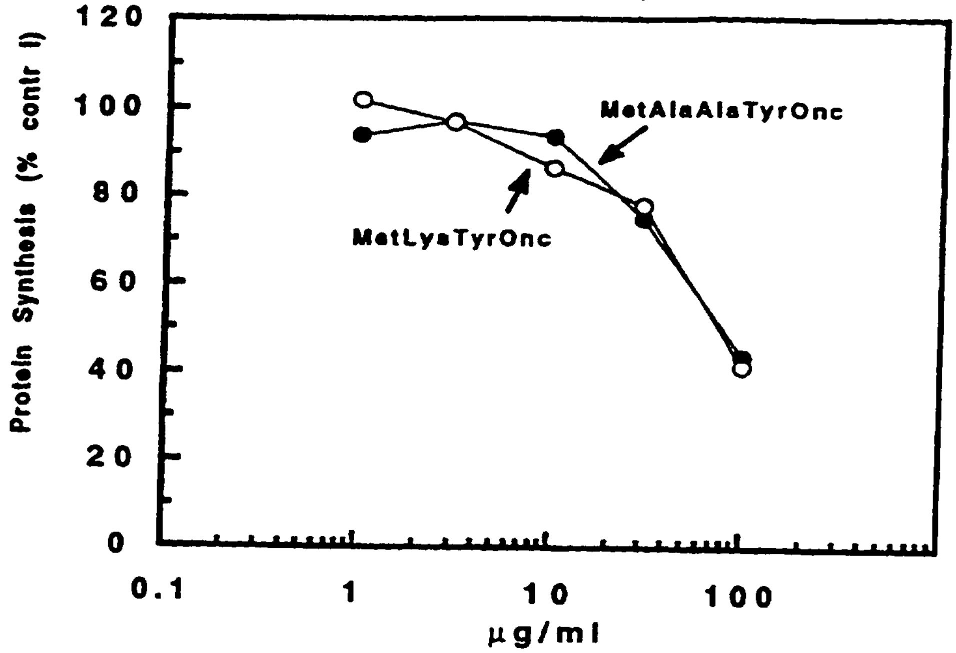


Figure 6A

Inhibition of cell viability by NLS-Onc and KDEL-Onc

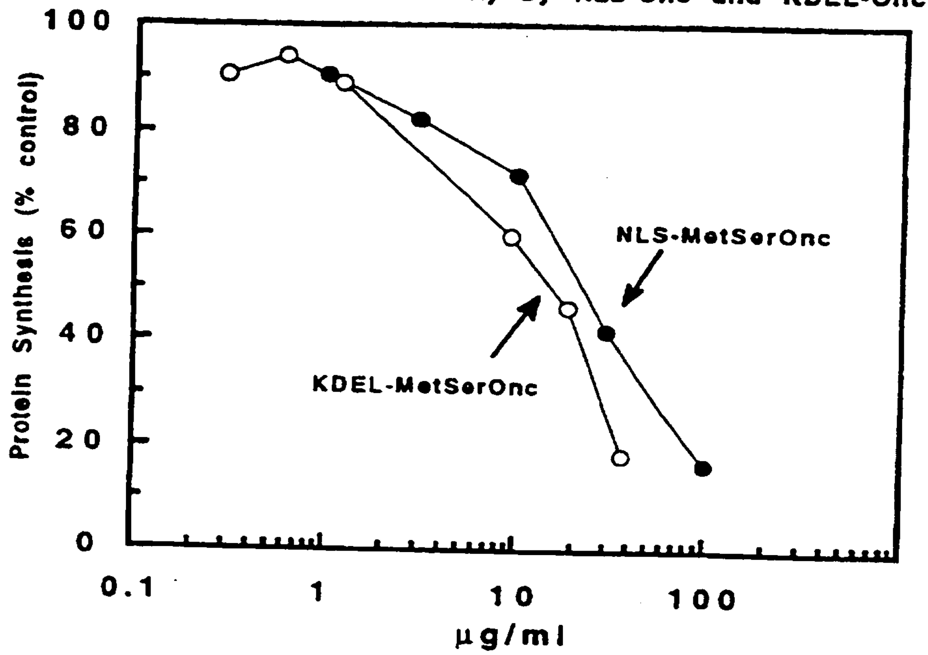


Figure 6B

Inhibition of Protein Synthesis by MetSerOnc or modified MetSerOncs containing cysteines

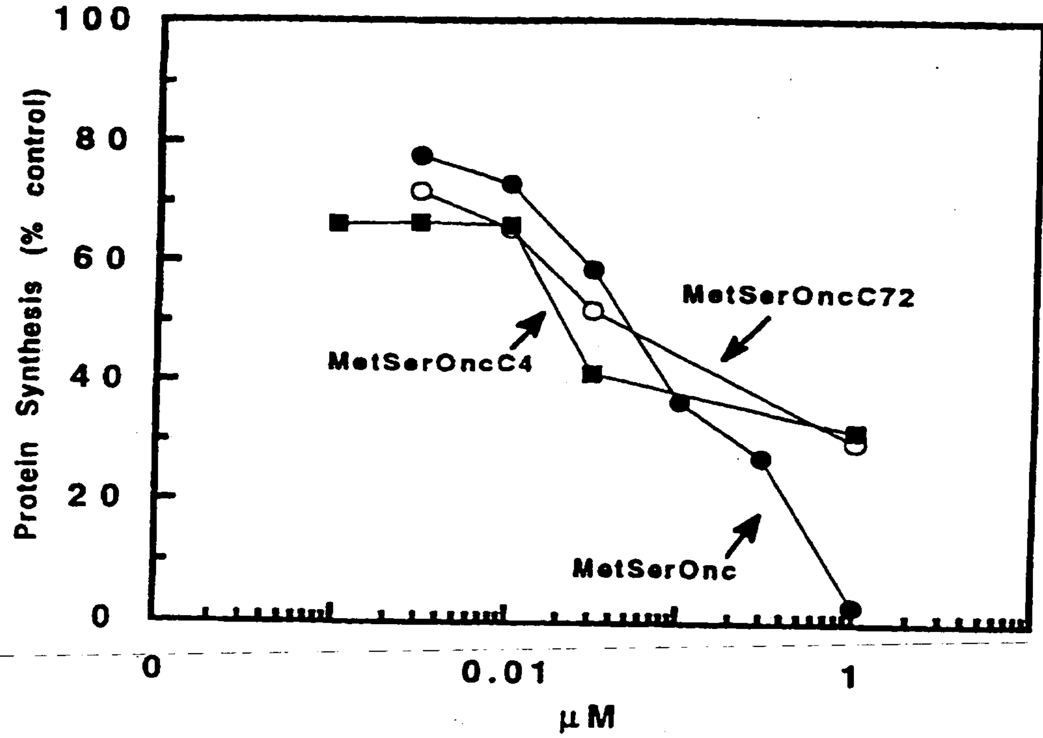


Figure 7

PCT

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(57) Abstract

The invention relates to ribonucleases derived from a native ribonuclease found in the oocytes of *Rana pipiens*. Various humanized and recombinant forms of these molecules are described as well as uses for them.

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INTERNATIONAL SEARCH REPORT

Intern[illegible]nal Application No
PCT/US 97/02588

A. CLASSIFICATION OF SU[illegible]MATTER
IPC 6 C12N15/55 C12N9/22 C12N15/62 A61K38/46 C12N15/70
C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 1, 5 January 1991, MD US, pages 245-251, XP000172527 WOJCIECH ARDELT ET AL.: "Amino acid sequence of an anti-tumor protein from Rana pipiens oocytes and early embryos"	22
A	see abstract	1-21, 23-32

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1997	08.08.97
Name and mailing address of the ISA	**Authorized officer**
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DRUG DELIVERY (1993), 1(1), 3-10 CODEN: DDELEB;ISSN: 1071-7544, 1993, XP000675395 RYBAK, SUSANNA M. ET AL: "Cytotoxic onconase and ribonuclease A chimeras: comparison and in vitro characterization" see abstract see page 4, left-hand column, paragraph 4 - right-hand column, paragraph 1 ---	1,23,24, 26,27
A	PROTEINS, vol. 14, no. 3, November 1992, pages 392-400, XP000675464 STEVEN C. MOSIMANN ET AL.: "Comparative molecular modeling and crystallization of P-30 protein: a novel antitumor protein of Rana pipiens oocytes and early embryos" see abstract see page 392, right-hand column, last paragraph - page 393, left-hand column, paragraph 1 see page 393, right-hand column, paragraph 2 - page 394, left-hand column, paragraph 2 see page 396, right-hand column, paragraph 3 - page 398, right-hand column, paragraph 4 -----	1

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INTERNATIONAL SEARCH REPORT

rnational application No.

PCT/US 97/02588

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 28, as far as in vivo method is concerned is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.